FINAL REPORT

15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley CD® Rats

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15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley (CD®) Rats

ABSTRACT

This study was done as a pre-validation step toward validating the intact adult male rat assay as an alternative Endocrine Disruptor Tier I screening assay (EDSTAC, 1998). The transferability or standardization of the protocol and the practicality or sensitivity of this *in vivo* assay was evaluated using Linuron and Methoxychlor, two chemical compounds known to affect the endocrine system through different pathways and/or mechanisms of action. This assay is expected to detect estrogenic-, androgenic- and thyrotropic-like activity based on compound-related changes in target organ weight and systemic hormones.

Animals were dosed daily for 15 days from SD 0 to 14 via oral gavage with respective chemical compounds at respective dose levels. Dose levels were administered on a mg/kg body weight basis according to the most recent body weight. Aqueous methylcellulose (0.25%) was administered as the vehicle control. For all animals, the dosing volume was 5 ml/kg and all animals were dosed between 0700 and 1000 hours.

Body weights were recorded on SD 0 through 13 before dosing and clinical observations were recorded daily within two hours after dosing in the morning and again in the afternoon. Food consumption was determined for SD 0, 7, and 14.

Necropsy was performed on SD 14 between 0700 and 1000 hours within two hours of final dosing. Blood was collected from anesthetized animals by cardiac puncture, target organs were collected and weighed and histopathology was done on the testes, epididymides and thyroids from the animals in the control and high dose groups.

The study design for the experiment was as follows for the two chemicals, linuron and methoxychlor:

Table 1. Study Design, Test Chemicals, and Target Doses

Group No.	No. Males	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
1	15	Vehicle Control[a]	0	0.0	5
2	15	Linuron	25	5.0	5
3	15		50	10.0	5
4	15		75	15.0	5
5	15		100	20.0	5
6	15	Methoxychlor	12.5	2.5	5
7	15		25	5.0	5
8	15		37.5	7.5	5
9	15		50	10.0	5

[a] 0.25% aqueous methylcellulose

- **Linuron**

  There were no deaths on the study. There was no significant difference in the mean body weights across all groups on SD 0. On SD 7, there were significant decreases in body weights of the 75 and 100 mg/kg/day groups compared to the control group at the $p<0.001$ level and 50 mg/kg/day at the $p<0.01$ level. The weight differences displayed a significant linear decreasing trend related directly to dose levels. At SD 14, the body weights of the control group were significantly higher than those of all the treated groups ($p<0.001$, and $p< 0.05$ for the 25 mg/kg/day group). The body weight changes for the treated groups were all significantly decreased in a dose related manner ($p<0.001$).

  Feed consumption (both g/day and g/kg/day) of the three highest treated groups was significantly decreased on SD 0-7 from the control group at the $p<0.001$ level and continued to be decreased throughout the remaining days of the study. The 25 mg/kg/day group showed a decreased feed consumption on SD 0-7 ($p< 0.05$) but no difference from the control group on SD 7-14 for either g/day or g/kg/day. Feed efficiency for SD 0-7 was significantly ($p<0.001$) decreased for the three highest dose groups, but was not significantly decreased for SD 7-14 except for the 50 mg/kg/day group at the $p< 0.05$ level (Table 4-A).

Clinical observations of piloerection, ataxia, lethargy, chromodacryorrhea, rough coat, afflux of the dosing solution, salivation prior to dosing, and rapid heart beat were made during the course of the study. Except for one animal with piloerection, only treated males had clinical signs. Lethargy was only seen in the highest dose group. Sores were seen in one animal.

At necropsy, the body weights of the animals in all groups but the low dose were significantly decreased from those of the control group ($p < 0.001$). The liver and accessory sex gland unit absolute weights of the three highest dose groups were significantly lower than those of the control group. There were no differences found in absolute paired testes weights between the control and treated groups and the absolute paired epididymides weights were significantly lower only in the highest dose group. The absolute prostate weights of the two highest dose groups were significantly lower than those of the control group. The ANOVA and test for linear trend were significant for the body weight, liver weight, and accessory sex gland unit weight at the $p < 0.001$ level.

When relative weights (% of terminal body weight) were considered, only the paired testis weights showed any significant differences. The relative paired testes weight of the 75 mg/kg/day group was significantly higher than the control group value and also appeared higher than the other treated groups, although this was not analyzed.

The serum estradiol level was significantly higher than the controls in all treated groups. This showed a dose related trend. Thyroxine level was lower in all treatment groups from the control. Prolactin was significantly lowered in the highest treatment group. Although all prolactin values in treated groups were lower than those of the control group values, there was no significant difference and no dose related response. Serum testosterone, DHT, FSH, LH, TSH, and T3 values were similar in all groups.

In the gross necropsy findings, the highest dose level had two males with bilateral testicular size reduction and one of these males had bilateral epididymis size reduction. The 75 mg/kg/day group had one animal with reduced prostate size and reduced size of seminal vesicles. The 50 mg/kg/day group had one animal with reduced epididymal size bilaterally and reduced testicular size bilaterally. No treatment-related histopathological changes were observed in the testes, epididymides, or thyroid. One animal, in the high dose group, was noted to have seminiferous tubule degeneration which was graded as moderate. However, in the experience of the reviewing pathologist, similar lesions may be, on occasion, observed in control animals as well.

♦ **Methoxychlor**

There were no deaths on the study. There was no significant difference in the mean body weights across all groups on SD 0. By SD 7, after the administration of methoxychlor, males in the three highest dose levels had significantly decreased body weights compared to the control group and this observation continued throughout the remainder of the treatment period for all the treated groups. The 12.5 mg/kg/day group did not have a significantly decreased body weight on SD 7 but did show a significant decrease by SD 14 by t-test ($p < 0.05$). The body weight changes between SD 0 to 7, 7 to 14, and 0 to 14, were all significantly lower for all of the treatment groups than for the control group. These changes did not show a dose-response effect.

The feed consumption (g/day and g/kg/day) was lower for all treated groups than the control group for all intervals, SD 0 to 7, 7 to 14, and 0 to 14. Feed efficiency was lower for the treated groups than the control group for the 0 to 14 interval.

Animals treated with methoxychlor showed clinical signs of chromodacryorrhea, efflux of the dosing solution, soft feces, piloerection (most common sign seen), rough coat, and rust colored fur. Except for piloerection, the incidence is only one animal.

At necropsy, there were significant decreases in all dose groups in body weight and absolute liver weight. The decreases in liver weights were also significant by ANOVA at the $p < 0.001$ level. The two highest dose groups had significantly lower absolute weights for the accessory sex gland unit, prostate and seminal vesicles with coagulating glands. The 25 mg/kg/day group also had significantly reduced absolute weights for the sex accessory unit and seminal vesicles. Relative weights (% of sacrifice weight) were decreased for the thyroid in all treated groups; and the relative weights of the accessory sex gland unit and the seminal vesicles with coagulating glands were reduced significantly in the highest dose group. The relative paired testis weight was significantly increased in the 37.5 and 50.0 mg/kg/day groups.

The mean serum prolactin levels of the 25 and 50 mg/kg/day groups were significantly higher ($p < 0.05$) than the control group value. There was also an apparent, but not significant, increase at 37.5 mg/kg/day. The mean thyroxine level of the of the 25 mg/kg/day group was higher than the control. Serum testosterone, DHT, FSH, LH, TSH, and T3 values were similar in all groups including control. Serum estradiol values

increased slightly with increasing dose levels but the differences were not statistically significant.

Administration of 50 mg/kg/day methoxychlor was associated with the increased incidence of testicular seminiferous tubule degeneration in 40% of the animals examined (6/15). Seminiferous tubule degeneration was characterized by a spectrum of very subtle changes which included vacuolization within the germinal epithelium lining the tubule, single cell degeneration to necrosis of germinal epithelial cells, desquamated germ cells into the tubule lumen and, on occasion, sperm retention in stage IX. At this dose, all of the degenerative lesions were graded as minimal which indicated they were present in fewer than 10% of the tubules present in any testis cross-section. In support of seminiferous tubule degeneration, increased numbers of exfoliated germ cells were present in epididymal tubules. Normally, low numbers of exfoliated germ cells may be observed in epididymal tubules, but these numbers can increase with seminiferous tubule degeneration. The pathogenesis and significance of the seminiferous tubule degeneration remains unclear at this time.

In conclusion:

- Fifteen days of gavage with Linuron resulted in decreased body weight, decreased feed consumption, decreased feed efficiency, and clinical signs such as piloerection and lethargy. Decreased sacrifice body weight, liver weight, accessory sex gland unit weight, and prostate and seminal vesicles with coagulating glands weights were observed at higher doses. The estradiol values increased with increasing doses whereas the thyroxine decreased. Prolactin was decreased for the 100 mg/kg/day group.

- Fifteen days of gavage with methoxychlor resulted in decreased body weight, decreased feed consumption, decreased feed efficiency, and clinical signs such as piloerection. Methoxychlor caused lower sacrifice body weights, liver weights, accessory sex gland unit weights, prostate, and seminal vesicles with coagulating glands weight. The prolactin level of the 50 mg/kg/day group was over twice that of the control group.

- The protocol for the adult male assay resulted in significant findings that appeared to be dose related for both linuron and methoxychlor.

OBJECTIVE

Purpose and Applicability

This study was done as a pre-validation step toward validating the intact adult male rat assay as an alternative Endocrine Disruptor Tier I screening assay (EDSTAC, 1998). The transferability or standardization of the protocol and the practicality or sensitivity of this *in vivo* assay was evaluated using Linuron and Methoxychlor, two chemical compounds known to affect the endocrine system through different pathways and/or mechanisms of action. This assay is expected to detect estrogenic-, androgenic- and thyrotropic-like activity based on compound-related changes in target organ weight and systemic hormones.

Required Endpoints:

- Daily body weights and sacrifice weights
- Serum Triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), prolactin, testosterone, estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), and dihydrotestosterone (DHT)
- Thyroid weights and histology
- Epididymal and testis weights and histology
- Liver, and accessory sex gland unit weights (including prostate, and seminal vesicle and coagulating gland weights)

To further define the procedures for this protocol, EPA tested two chemical compounds that have various modes of action:

- Linuron (anti-androgen),
- Methoxychlor (estrogenic, anti-estrogenic and anti-androgenic activity).

MATERIALS AND METHODS

Test Materials and Dose Formulations

The test chemicals used in this study were procured and analyzed for purity by the Chemical Repository at Battelle, Sequim, WA, by gas chromatography with flame ionization detection (GC-FID), or high performance liquid chromatography (HPLC) methods. All bulk test chemicals were stored at room temperature. Test chemicals formulated in 0.25% aqueous methylcellulose were stored at 4°C. Dose formulations were prepared by mixing each test material at the appropriate weight in 0.25% aqueous methylcellulose to provide the appropriate mg/kg/day administration at 5 ml/kg. The

vehicle formulation was prepared and administered to the control group animals concurrently with the administration of the two test compounds to the remaining animals. Formulations were prepared at the RTI Materials Handling Facility, and assayed at Battelle, Sequim, WA.

The decision was made to formulate the doses of linuron and methoxychlor at RTI International. The formulations were mixed three separate times and sent to Battelle-Sequim for analysis of the formulations and homogeneity determination. In-life samples were collected at the beginning and end of the time period that the formulations were used for dosing.

Samples were only collected from the two middle dose groups of linuron (10 and 15 mg/mL) and methoxychlor (5.0 and 7.5 mg/mL) due to a misinterpretation of the directions in the protocol by the technical staff. Samples should have been taken from all dose groups during the in-life dosing. The samples were collected at the conclusion of dosing on the days of subsampling.

Animals and Husbandry

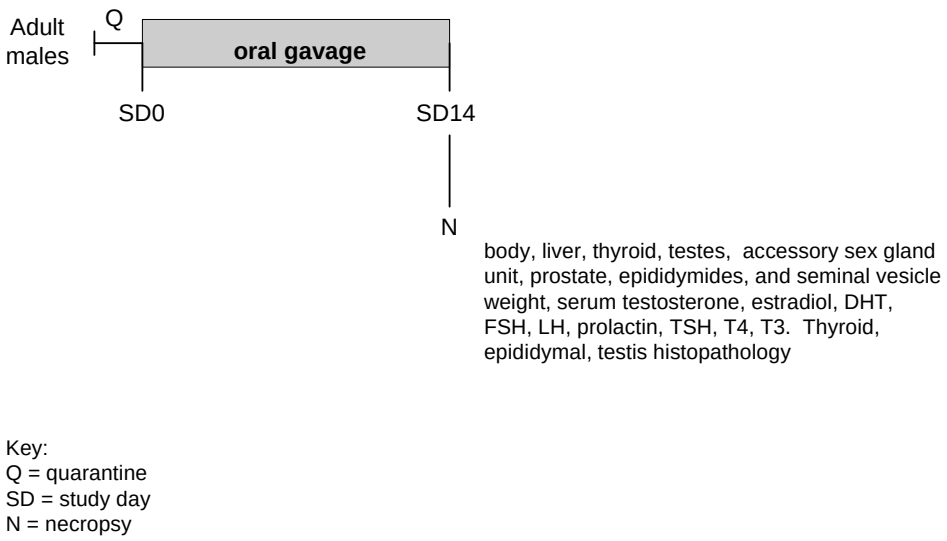
Male outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD] IGS BR) were received from Charles River Breeding Laboratories (Raleigh, NC). The males were approximately 10 weeks old upon arrival. The animals were individually housed during the quarantine period and experimental periods in solid-bottom polycarbonate cages with stainless-steel wire lids (Laboratory Products, Rochelle Park, NJ) and Sani-Chip® cage litter (P.J. Murphy Forest Products Inc., Montville, NJ). The males were housed under standard conditions until necropsy. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 14:10 hour (light:dark) light cycle per day and were air-conditioned. Temperature (22±4°C) and relative humidity (30-70%) were continuously monitored, controlled, and recorded using an automatic system (Siebe/Barber-Colman Network 8000 System, Version 4.4.1, Loves Park, IL). Purina Certified Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO) and deionized water were available *ad libitum*.

Adult male rats were individually identified by eartag. A total of 15 males per group was assigned to each component in this study. Males were assigned to treatment groups by stratified randomization for body weight upon arrival at RTI International so that the mean body weights did not differ among treatment groups at the start of dosing. They were examined twice daily by cage-side observation for morbidity or mortality and clinical observations occurred initially within two hours after dosing and also in the afternoon by the animal husbandry staff. All animals assigned to the study were euthanized by CO_2 asphyxiation after 15 days of dosing.

Additional Methods. Feed consumption was measured on SD 0, 7, and 14. The feed was also analyzed by the manufacturer for the phytoestrogens daidzein, genistein, and glycitein (Appendix III).

The singly housed animals, collection of feed consumption data, and the analysis of phytoestrogens in the feed were additions to the basic study design.

Study Design for the Adult Male Assay



Clinical observations of adult male study animals were documented at least once daily during quarantine and at least twice daily, at dosing and one to two hours post dosing, on SD 0 through 13. On SD 14, cage side observations were made at dosing . All males were weighed every morning SD 0 through 13, for adjustment of dosing volume based on the most recent body weight. The dosing volume for SD 14 dosing was based on the body weight of SD 13. Daily body weights and body weight gains were reported and statistically analyzed (Tables 3A, 3B).

Necropsy for Males

Blood Collection and Hormone Assays

At scheduled necropsy of the males, after terminal CO_2 asphyxiation, the males were weighed and the maximum possible amount of blood was taken by external cardiac puncture and placed in a labeled serum separator tube on ice. The blood was allowed to clot and centrifuged under refrigeration. The resulting serum was analyzed for T4, T3, TSH, FSH, LH, testosterone, DHT, estradiol, and

prolactin. All assays were counted in a Packard Biosciences Cobra II Series Model 5002 gamma counter using RIASMART software, version 1.0.

Estradiol Radioimmunoassay Procedure

The estradiol radioimmunoassay (RIA) used was a no-extraction, double antibody ^{125}I RIA (Diagnostic Systems Laboratories [DSL], Webster, Texas) which utilized estradiol antibody, ^{125}I-estradiol, estradiol calibrators as the standard curve, and a precipitating solution consisting of goat anti-rabbit gamma globulin combined with dilute polyethylene glycol. Normal control serum from the same species/strain/sex as unknown samples was assayed. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.6 pg/mL as reported by DSL. All samples read within curve range of 1.5 to 150 pg/mL. For the RIA procedure, the sample (200 µL) was pipetted into a glass culture tube and the estradiol antiserum was added. The tubes were vortexed and incubated at 4°C for 4 hours. The ^{125}I-estradiol was added, and the tubes were vortexed and incubated at 4°C for 21 hours. After overnight incubation, cold precipitating solution was added and the tubes vortexed. The tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as pg/mL.

Rat Follicle Stimulating Hormone Radioimmunoassay Procedure

The rat follicle stimulating hormone (rFSH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rFSH antibody, ^{125}I-rFSH, rFSH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-sheep serum coated onto magnetizable polymer particles. Normal control serum from the same species/strain/sex as unknown samples was assayed. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.9 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 1.6 to 100 ng/mL. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube and the rFSH antiserum was added. The tubes were vortexed and incubated at room temperature for 4 hours. The ^{125}I-rFSH was added, and the tubes were vortexed and incubated at room temperature for approximately 20 hours. After overnight incubation, cold precipitating solution was added and the tubes vortexed. The tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL.

Rat Luteinizing Hormone Radioimmunoassay Procedure

The rat luteinizing hormone (rLH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rLH antibody, ^{125}I-rLH, rLH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-rabbit serum coated onto

magnetizable polymer particles. Normal control serum from the same species/strain/sex as unknown samples was assayed. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.8 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 0.8 to 50 ng/mL. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rLH antiserum was added, followed by the ^{125}I-rLH, and the tubes were vortexed and incubated at room temperature for approximately 22 hours. After overnight incubation, cold precipitating solution was added and the tubes vortexed. The tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL.

Rat Prolactin Radioimmunoassay Procedure

The rat prolactin (rPRL) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rPRL antibody, ^{125}I-rPRL, rPRL calibrators as the standard curve, and a precipitating solution consisting of donkey anti-sheep serum coated onto magnetizable polymer particles. Normal control serum from the same species/strain/sex as unknown samples was assayed. From the standards values the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.7 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 0.8 to 50 ng/mL, once diluted 10-fold with assay buffer. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rPRL antiserum was added, followed by the ^{125}I-rPRL, and the tubes were vortexed and incubated at room temperature for 20 - 21 hours. After overnight incubation, cold precipitating solution was added and the tubes vortexed. The tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL.

Rat Thyroid Stimulating Hormone Radioimmunoassay Procedure

The rat thyroid stimulating hormone (rTSH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rTSH antibody, ^{125}I-rTSH, rTSH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-rabbit serum coated onto magnetizable polymer particles. Normal control serum from the same species/strain/sex as unknown samples was assayed. From the standards values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.5 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 1 to 64 ng/mL. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rTSH antiserum was added, followed by the ^{125}I-rTSH, and the tubes were vortexed and incubated at room temperature for approximately 21 hours. After overnight incubation,

cold precipitating solution was added and the tubes vortexed. The tubes were centrifuged, the supernatant was decanted, and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL.

Total Testosterone Radioimmunoassay Procedure

The total testosterone (T) RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T-specific antibody-coated tubes and ^{125}I-T (Diagnostic Products Corporation [DPC], Los Angeles, CA). The T (Sigma, St. Louis, MO) standard curve was prepared in PBS-gel buffer (0.1 M sodium phosphate plus 0.85% [w/v] sodium chloride with 0.1% [w/v] sodium azide and 0.1% [w/v] gelatin, pH 7.4). T standards in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of T to the appropriate matrix. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.04 ng/mL as reported by DPC. All samples read within curve range of 0.07 to 40 ng/mL. For the RIA procedure, the sample (50 µL) was pipetted into the antibody-coated tube and the ^{125}I-T was added. The tubes were vortexed and incubated in a 37°C water bath for three hours. After incubation, the supernatant was aspirated and the tubes were counted in a gamma counter. Results were reported as ng/mL.

Total Triiodothyronine Radioimmunoassay Procedure

The total triiodothytonine (T3) RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T3-specific antibody-coated tubes and ^{125}I-T3 (DPC, Los Angeles, CA). The T3 (Sigma, St. Louis, MO) standard curve was prepared in charcoal stripped serum. T3 controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of T3 to the appropriate matrix. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 7 ng/dL as reported by DPC. All samples read within curve range of 6.25 to 800 µg/dL. For the RIA procedure, the sample (100 µL) was pipetted into the antibody-coated tube and the ^{125}I-T3 was added. The tubes were vortexed and incubated in a 37°C water bath for two hours. After incubation, the supernatant was aspirated and the tubes were counted in a gamma counter. Results were reported as ng/dL.

Total Thyroxine Radioimmunoassay Procedure

The total thyroxine (T4) RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T4-specific antibody-coated tubes and ^{125}I-T4 (DPC, Los Angeles, CA). The T4 (Sigma, St. Louis, MO) standard curve was prepared in RIA Buffer I (0.01 M sodium phosphate plus 0.85% [w/v] sodium chloride with 0.1% [w/v] sodium azide and 1% [w/v] bovine serum albumin, pH 7.6). T4 standards in serum were prepared in the same species/strain/sex as unknown samples by adding known

concentrations of T4 to the appropriate matrix. From the control values, the intra- and interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Text Table 2). The sensitivity of the assay was 0.25 μg/dL as reported by DPC. All samples read within curve range of 0.63 to 40 μg/dL. For the RIA procedure, the sample (25 μL) was pipetted into the antibody-coated tube and the ^{125}I-T4 was added. The tubes were vortexed and incubated in a 37°C water bath for one hour. After incubation, the supernatant was aspirated and the tubes were counted in a gamma counter. Results were reported as μg/dL.

Dihydrotestosterone Radioimmunoassay Procedure

The dihydrotestosterone (DHT) RIA used had a sample oxidation/extraction procedure followed by a solid-phase ^{125}I RIA which utilized DHT-specific antibody-coated tubes, ^{125}I-DHT, and DHT standards (DSL, Webster, Texas). Also included in the kit were reagents for the oxidation/extraction procedure to remove most of the testosterone which will cross-react with the DHT antiserum. These reagents were an oxidation solution and DHT sample buffer. Also needed but not included were the organic solvents for extraction, n-hexane (95% minimum) purchased from EM Science, and absolute ethanol which was purchased from AAPER Alcohol and Chemical Company. DHT standards in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of DHT to the appropriate matrix. From the control values, the intra- and interassay coefficient of variation, and percent recovery for the assays was determined (see Text Table 2). The sensitivity of the assay was 4 pg/mL as reported by DSL. All samples read within curve range of 25 to 2500 pg/mL. For the RIA procedure, the sample (400 μL) was extracted and then pipetted into the antibody-coated tube and the ^{125}I-DHT was added. The tubes were vortexed and incubated at room temperature on a shaker (180 rpm) for two hours. After incubation, the supernatant was decanted and the tubes were counted in a gamma counter. Results were reported as pg/mL.

Gross Examination and Histopathology

Once each male was bled, the animal was necropsied and internal thoracic and abdominal organs and cavities examined. Any abnormalities were documented. The following organs were dissected out and weighed: paired testes, paired epididymides, liver, thyroid (taken with attached portion of trachea, weighed after fixation and removal of the tracheal portion), accessory sex gland unit [prostate and seminal vesicles with coagulating glands (and fluid)], and prostate and seminal vesicles with coagulating glands (with fluid).

Table 2. Parameters for RIAs Used for Adult Male Hormone Determinations

Parameter	Testosterone	Estradiol	rFSH	rLH	rTSH	T4	T3	rProlactin	DHT
Units	(ng/mL)	(pg/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ug/dL)	(ng/dL)	(ng/mL)	(pg/mL)
Intra-assay Variation[a]									
BLANK MATRIX	n/a	0/9.8%	0/2.9%	0/15.8%	0/7.6%	0/3.2%	n/a	0/4.3%	0/8.6%
MASS ADDED	2/13.4% 8/6.2%	20/8.6% 80/4.6%	12.5/4.2%	6.2/2.5% 25/3.9%	8/8.3%	2/6.2% 10/4.5%	40/12.3% 160/5.7%	3.1/6.1% 12.5/8.2%	100/13.6% 400/9.9%
Inter-assay Variation[a]									
NO. OF ASSAYS	1	1	1	2	1	1	2	2	2
BLANK MATRIX	n/a	n/a	n/a	0/8.1%	n/a	n/a	n/a	0/4.8%	0/18.2%
MASS ADDED	n/a	n/a	n/a	3.1/7.8% 12.5/3.2%	n/a	n/a	40/14.2% 160/2.6%	12.5/13.5%	100/18.9% 400/17.4%
% recovery of added mass[b]	2/78.0% 8/75.6%	20/142.5% 80/122.3%	12.5/94.7% 50/112.7%	3.1/(66.5%-70.0%) 12.5/(89.8%-92.1%	8/120.5%	2/73.0% 10/80.6%	40/(94.7%-101.0%) 160/(99.7%-102.4%)	3.1/117.1% 12.5(64.3%-105.1%)	100/75.1% 400/86.7%
Index of parallelism[c]	121.2%	20/95.7% 80/94.4%	106.1%	6.2/97.5% 25/98.1%	0/117.0% 8/96.4%	2/111.2% 10/112.6%	100.6%	0/75.5% 12.5/79.4%	n/a

[a] Numbers are mass added/percentage variation. For intra-assay variation, the number of samples assayed was 8-10 in each case.

[b] Numbers are mass added/percentage recovered (range of all assays).

[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.

n/a = not applicable

Tissues taken at necropsy were placed in fixative (10% neutral buffered formalin or Bouin's). After 24 hours, tissues placed in Bouin's fixative (testes) were rinsed and stored in 70% alcohol until embedded in paraffin. Tissues were transferred to Experimental Pathology Laboratories (EPL) Inc., Research Triangle Park, NC, for processing. The thyroids for the control and high dose groups were weighed at EPL, after removal of the trachea. The thyroids for the other treatment groups were weighed at RTI after removal of the trachea. The tissues were embedded in paraffin, sectioned at 3-5 microns, and stained with hematoxylin and eosin (H and E) for subsequent histopathological evaluations. Stained sections were evaluated for pathologic abnormalities and potential treatment-related effects by an AVCP board-certified veterinary pathologist. Only tissues for the control group and high dose linuron and high dose methoxychlor were evaluated.

Statistical Analyses

All data for a single chemical (four doses) and a concurrent vehicle control group were analyzed using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967). The homogeneity of variance assumption was examined via Levene's test (Levene, 1960). When Levene's test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for linear trends across dose as well as overall treatment group differences (via Wald chi-square tests). Significant overall treatment effects were followed by single degree-of-freedom *t*-tests for each treated group vs. control group comparisons, if the overall treatment effect was significant. When Levene's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) was used to test for linear trend, evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group to control via Dunnett's test (Dunnett, 1955, 1964). Standard ANOVA methods, as well as Levene's test, are available in the GLM procedure of SAS®, and the robust regression methods were available in the REGRESS procedure of SUDAAN® Release 7.5.4 (Shah *et al*., 1997) or Release 8.0 (RTI, 2001). Organ weights were also analyzed by Analysis of Covariance (ANCOVA) using the body weight at necropsy as the covariate. When statistically significant effects were observed, treatment means were examined further using LSMeans.

A test for statistical outliers was performed in the UNIVARIATE procedure of SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) on male body and organ weights. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. For all statistical tests, $p \leq 0.05$ (one- or two-tailed) was used as the criterion for significance.

Personnel

This study was conducted at RTI International, Research Triangle Park, NC, under contract to Battelle, Columbus, OH. Dr. David P. Houchens, EDSP Program Manager, was the Sponsor's Representative. Dr. R.W. Tyl served as Project Toxicologist. Dr. J. D. George served as Work Assignment Leader and Carol D. Sloan as Study Director. Reproductive and Developmental Toxicology and Laboratory of Reproductive and Endocrine Toxicology personnel included Ms. M.C. Marr , Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W. P. Ross, Ms. V.I. Wilson, Ms. L.B Pelletier, Ms. N.M. Kuney, Ms. R.T. Krebs, Ms. S.W. Pearce, Ms. K.D. Vick, Ms. L. McDonald, Ms. A. J. Parham, Mr. M.D. Crews, Mr. C.G. Leach, Ms. A. B. Goodman, and Mr. T.W. Wiley. Bulk chemical analysis and dose formulation analysis were provided by the Sponsor through Dr. E.A. Crecelius, PNNL, Battelle Marine Sciences Laboratory, Sequim, WA. Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, Mr. J. Larson, and Mr. R.A. Price provided receipt of the bulk chemicals and preparation of dose formulations at RTI. Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, RTI Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility. RTI Quality Assurance personnel were Ms. D.A. Drissel (Manager), Ms. D.J. Smith, Ms. M.D. Phillips, Ms. T.M. Kenney, and Ms. C. Ingalls. Contractors Ms. M. Oh and Mr. Steven Myers, audited the hormone data and analyses. Dr. G. Waterhouse audited the draft final report.

The final report was prepared by Ms. C.D. Sloan, with assistance from Dr. R.W. Tyl, Dr. J.D. George, Ms. B. Hamby, Ms. C.B. Myers, Ms. K.D. Vick, Ms. S.W. Pearce, and Ms. M.C. Marr. Ms. C.B. Myers was responsible for data compilation, statistical analyses and table generation, and Mr. T.W. Wiley was responsible for data entry. Ms. M.C. Marr and Ms. K.D. Vick were responsible for all activities concerning organization and custody of the study records and for archiving the study records. Ms. D. Bynum and Ms. K. Kehagias provided secretarial assistance.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the period specified by the contract or for as long as the quality of the preparation affords evaluation, whichever is less. Quality control (QC) and quality assurance (QA) procedures followed those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study and in accordance with the Quality Management Plan (QMP) for this project. The RTI Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. Animals were housed, handled, and used according to the NRC Guide (NRC, 1996).

RESULTS

Chemistry

Two different lots of linuron were received by Battelle-Sequim and purity was determined on both lots. The purity of Lot No. 273-81B was 99.6% and Lot No. 301-91A was 99.9%. Lot No. 273-81B was used for stability testing and Lot No. 301-91A for biological testing. Stability testing of the linuron stock solution in methylcellulose was considered stable at 5 mg/mL for 21 days.

The in-life linuron concentrations had a mean of 7.19 for the 10 mg/mL target and 15.4 mg/mL for the 15 mg/mL target. Recoveries relative to the dosing target ranged from 31% to 130% for the 10 mg/mL target and 40% to 174% for the 15 mg/mL target concentration.

The purity of the methoxychlor (Lot No. 049H1328) was determined to be 89.99% by Battelle-Sequim, although the manufacturer had stated a purity of 95.2%. The stability was estimated for up to 16 days for 2.5 mg/mL in methylcellulose.

The in-life methoxychlor concentrations had a mean of 5.26 mg/mL for the 5.0 mg/mL and a range of recovery of 73-161% of the target. The mean for the 7.5 mg/mL target concentration was 7.88 mg/mL and the range of recovery was 49 to 139% of the target.

The linuron purity, stability, and homogeneity results were acceptable. The in-life concentrations for the 15 mg/mL target was acceptable as far as the mean values were concerned but the ranges were very large. The mean for the 10 mg/mL target was very low and also had too large a range of values.

Linuron in methylcellulose resulted in a suspension that was difficult to keep well mixed during dosing and therefore, both dosing and sampling may not have been as accurate as would have been desired.

The methoxychlor purity was below 90%. The stability and homogeneity were acceptable for methoxychlor. The mean values of the in-life samples were very close to the target concentrations of 5.0 and 7.5 mg/mL but the ranges were very large.

Formulations of Linuron and Methoxychlor

The wide ranges of concentrations of the formulation in-life samples are of concern. This variation was probably due to a combination of factors. The suspensions in methylcellulose were extremely difficult to keep suspended even with constant vortexing. Technicians began vortexing the formulations at least an hour before dosing and kept vortexing the formulations while dosing and taking

subsamples. Since the biological responses were consistent with previous studies using antiandrogens, RTI believes that the animals did receive an overall mean of the concentrations, although the daily concentrations may have varied more than desired.

The concentrations of linuron and methoxychlor did not vary as much as in other studies conducted in our laboratory where these substances were mixed with corn oil. Perhaps that would have been preferable for the adult male assay also but one of the objectives of this study was to duplicate the chemical industry adult male protocol which used methylcellulose.

STUDY RESULTS

Control Males

Fifteen intact adult males were assigned to the control group and gavaged daily with the 0.25% aqueous methylcellulose vehicle.

In-Life Data From Males Treated With Linuron

Fifteen intact adult males were assigned to the 25, 50, 75, and 100 mg/kg/day linuron groups. There were no animal deaths on study. Body weights were taken daily and statistical analysis performed on weights from SD 0, 7 and 14 (Table 3-A). By SD 14, daily body weights for males were significantly decreased in all dose groups compared to the control group. Body weight change was significantly less in linuron-treated groups compared to the control group and this linear trend response was significant as well as the ANOVA (Table 3-A).

Feed consumption (both g/day and g/kg/day) of the three highest treated groups was significantly decreased on SD 0-7 from the control group at the $p<0.001$ level and continued to be decreased throughout the remaining days of the study. The 25 mg/kg/day group showed a decreased feed consumption on SD 0-7 ($p< 0.05$) but no difference from the control group on SD 7-14 for either g/day or g/kg/day. Feed efficiency for SD 0-7 was significantly ($p<0.001$) decreased for the three highest dose groups, but was not significantly decreased for SD 7-14 except for the 50 mg/kg/day group at the $p< 0.05$ level (Table 4-A).

Clinical observations were noted in the linuron-treated groups, and included ataxia, chromodacryorrhea, efflux of the dosing solution, rapid heart beat, lethargy, piloerection, rooting post dosing, rough coats, salivation prior to dosing, and malocclusion and appeared to be dose related. Sores were seen in one animal (Table 5-A). The only observation in control animals was 1 male with piloerection. The number of animals with clinical observations increased with increasing doses of linuron.

Necropsy, Hormone and Histopathological Data from Males Treated with Linuron

At necropsy, average body weight exhibited a dose-related decreasing trend, with values from the three highest linuron-treated groups significantly below the control group value (Table 6-A).

At necropsy, the mean thyroid weight (trimmed and weighed at EPL) of the high dose treatment group and control were similar. Absolute thyroid weights for the other dose groups, trimmed and weighed at RTI, were significantly lower than the control group. The values for the control group and highest dose level group were similar. The mean liver weights and accessory sex gland unit weights of the three highest treated groups were significantly lower than the mean weight of the control group. There were no differences found in the mean paired testes weights between the control and treated groups and the mean paired epididymides weights were significantly lower only in the highest dose group. The prostate weights of the two highest doses were significantly lower than the control group (Table 6-A). The seminal vesicle and coagulating gland weights were significantly lower in the 100 and 50 mg/kg/day groups than the control.

When relative weights were considered (% of terminal body weights), only the thyroid and the paired testis weights showed any significant differences. The relative paired testis weight of the 75 mg/kg/day group was significantly greater than the control (Table 6-A). The analysis of covariance with body weight at sacrifice as a covariate was significant for the thyroid, because of the trimming differences, and a linear trend was seen in the decreasing weights of the prostate and accessory sex gland unit weight.

Blood was collected by cardiac puncture from all rats at the time of sacrifice and serum analyzed for hormone levels. The circulating levels for serum testosterone, dihydroxytestosterone (DHT), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and triiodothyronine (T3) were similar for both control and treated groups (Table 7-A). Estradiol values were higher in all treated groups and showed a dose related increasing linear trend. Thyroxine (T4) levels were significantly lower in all treatment groups compared to the control group. Prolactin values were apparently lower than the controls in all treated groups, but the differences were statistically significant only in the 100 mg/kg/day group (Table 7-A).

Gross necropsy findings were minimal, and included one animal in the 50 mg/kg/day group with reduced paired testicular and epididymal size bilaterally, one animal in the 75 mg/kg/day group with the prostate and seminal vesicles decreased in size and two animals in the 100 mg/kg with the paired testes size reduced; one of these also had reduced epididymal size bilaterally.

Histopathology was conducted on tissues in the control and high dose treated group. No treatment associated lesions were observed in the linuron exposed animals. One animal in the high dose group did have seminiferous tubule degeneration graded as moderate but this has also been occasionally observed in control animals according to the examining pathologist.

In-Life Data from Males Treated with Methoxychlor

Fifteen males were assigned to the 12.5, 25, 37.5, and 50 mg/kg/day methoxychlor groups. No animals died during the study. There was no significant difference in the mean body weights in any dose group on SD 0. On SD 7, there were significant decreases in body weights of the 37.5, and 50 mg/kg/day groups from those of the control group at the $p<0.001$ level and the 25 mg/kg/day group at the $p<0.01$ level. The weight differences displayed a significant decreasing linear trend related directly to increasing dose levels. By SD 14, the mean body weights of the three highest treated groups were significantly lower than those of the control group value ($p<0.001$) and continued to display a dose-dependent downward linear trend. The body weight changes in treated males from SD 0-7, 7-14, and 0-14 were all significantly lower than the control group and there was a significant decreasing linear trend with increasing dose (Table 3-B).

The feed consumption of the treated groups was significantly decreased on SD 0 through 7 from the control group at the $p<0.001$ level, the 12.5 mg/kg/day was significant at the $p<0.01$ level, and continued to be decreased throughout the 15 days of the study (Table 4-B).

Clinical observations of piloerection, chromodacryorrhea, rough coats, rust colored fur, soft feces, and efflux of the dosing solution were made during the course of the study in the treated groups (Table 5-B). Except for piloerection, the incidence was only one animal.

Necropsy, Hormone and Histopathological Data from Males Treated with Methoxychlor

At necropsy, the mean thyroid weights of the control and high dose group (trimmed and weighed at EPL) were similar. Thyroid weights for the other dose groups, trimmed and weighed at RTI, were significantly lower than the control. The mean liver weights of the treated groups were significantly lower than the mean weights of the control group. The three highest treatment groups had significantly lower accessory sex gland unit weights (Table 6-B). There were no differences found in the mean paired testes weights between the control and treated groups and the mean paired epididymides weights were significantly lower only in the 37.5 mg/kg/day dose group. The prostate weights for the two highest treatment groups were significantly lower than the controls. The three highest treatment groups showed lower weights than the control group for the seminal vesicles with coagulating glands (with fluid).

The high dose relative thyroid weight was significantly increased compared to the control group. The relative paired testes weight of the 37.5 and 50 mg/kg/day groups were significantly greater than the control group value. The accessory sex gland unit and seminal vesicles with coagulating glands were significantly reduced at the 50 mg/kg/day concentration (Table 6-B).

The circulating levels for serum testosterone, dihydrotestosterone (DHT), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and triiodothyronine (T3) were similar for both control and treated groups (Table 7-B). Thyroxine (T4) levels were significantly higher in the 25 mg/kg/day treatment group (and unaffected at 50 mg/kg/day) compared to the control group. Prolactin values were higher in the 25 and 50 mg/kg/day groups than in the control group. The prolactin values in the 37.5 mg/kg/day group were increased but not significantly.

Gross necropsy findings were minimal, and included two animals in the 50 mg/kg/day methoxychlor group with reduced size of the prostate and seminal vesicles (Table 8-B). Histopathology was conducted on tissues from the control and high dose treated group. Administration of 50 mg/kg/day methoxychlor was associated with the increased incidence of testicular degeneration in 40% of the animals examined.

Seminiferous tubule degeneration was characterized by a spectrum of very subtle changes which included vacuolization within the germinal epithelium lining the tubule, single cell degeneration to necrosis of germinal epithelial cells, desquamated germ cells into the tubule lumen and, on occasion, sperm retention in stage IX. All the degenerative lesions were graded as minimal, which indicated they were present in fewer than 10% of the tubules present in any testis cross-section. Increased numbers of exfoliated germ cells were present in epididymal tubules. The significance and pathogenesis of the seminiferous tubule degeneration are unclear.

DISCUSSION AND CONCLUSION

This study was done as a pre-validation step toward validating the intact adult male rat assay as an alternative Endocrine Disruptor Tier I screening assay (EDSTAC, 1998). The transferability or standardization of the protocol and the practicality or sensitivity of this *in vivo* assay was evaluated using Linuron and Methoxychlor, two chemical compounds known to affect the endocrine system through different pathways and/or mechanisms of action. This assay is expected to provide a means of screening the effects of potential endocrine disruptors that may alter a number of endocrine-dependent mechanisms, including estrogenic-, androgenic-, and thyrotropic-like processes (Goldman et al., 2000). The endpoints in this study were chosen to reflect specific changes in general toxicity, reproductive and thyroid organ weights and systemic hormone concentrations, in part, in response to Linuron and Methoxychlor.

Linuron and Methoxychlor induced body weight decreases and histological changes in some reproductive organs.

The thyroid weights were similar for the control group and the linuron and methoxychlor highest dose groups, however, the lower dose groups for each chemical were lower than the control or highest dose groups. This difference is most likely due to the control and highest level dose groups being trimmed from the trachea and weighed at EPL where histology was conducted, whereas the other dose

groups were trimmed and weighed at a later time at RTI. This probably led to differences in trimming as well as some increased dehydration in the groups that were processed later leading to lower weights.

There was greater variation in the percent recovery of the linuron after dosing as compared to the methoxychlor. The linuron was very difficult to keep in suspension while dosing even with constant stirring while dosing. This fact combined with the delay in time from dosing completion to analysis may account for some of the variability.

The results show that the adult male assay with 15 days of dosing can show how these two chemicals affect the animals' body weights, weights of some organs and changes in the level of several of the hormones measured.

Linuron and methoxychlor are both antiandrogens. Linuron is a urea-based herbicide which has been shown to have a weak affinity for the androgen receptor. A multi-generation study with rats dosed with Linuron led to a range of male reproductive tissue problems in offspring, including testicular malformations and reduced size of androgen-dependent tissues (Gray et al., 1999). The present study showed that adult male rats dosed orally for 15 consecutive days would display decreased body weights and feed consumption. They also showed decreased liver, prostate, and seminal vesicle with coagulating glands weights. Hormone values showed increases in estradiol and decreases in thyroxine and prolactin.

Methoxychlor is a pesticide in the DDT family. A metabolite, HPTE, reduces testosterone production by Leydig cells by down regulation of one of four enzymes that catalyze reactions occurring during androgen biosynthesis. In the current study, the hormonal effect was the increased prolactin level in the 50 mg/kg/day group. Decreased body, liver, prostate, and seminal vesicle with coagulating glands weights were observed with methoxychlor as was seen with linuron.

This assay is an adequate screening assay when the markers of body, liver, prostate, and seminal vesicles with coagulating glands weights are used. The hormonal data may be too variable in this short duration of treatment time although differences were seen. The assay also limits the sacrifice times and length of dosing to time of sacrifice and therefore limits the number of animals sacrificed per day. Practically, this *in vivo* assay may be too limiting as a screening assay. Nonetheless, results indicated significant findings that appeared to be dose related for both linuron and methoxychlor.

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Deviations

SOP Deviations		
Deviation/SOP Number	**Effect on Study**	**Reason**
Data was not initialed and dated when technician returned to room to perform additional tasks/ TERA-602.3	None	Technician error
PM Observations were not initialed and dated	None	Technician error
Animal # 181 (Rx code 09560) received 2.25 ml instead of 2.20ml/ TERA-106.9	None	Technician error
Animal # 5 (Rx code 69300) received 1.90 ml instead of 1.95 ml/ TERA 106.9	None	Technician error
Animal #265 (Rx code 43804) received 2.40ml instead of 2.25ml/TERA-106.9	None	Technician error
Person dosing animal signed initials instead of recorder/TERA 602.3	None	Technician error
Not all PM observations were initialed since they were performed by the same technician as the AM checks	None	Technician error. Technicians did sign in on the room log and there was no designated space on the observation/dosing sheets for PM observations since they were added by the study director after significant lethargy was seen in the PM in some treated rats

Protocol Deviations		
Deviation	**Effect on Study**	**Reason**
The QC animals did not have serology done	None	The animals were only in house for about three weeks and serology is not required by the ARF SOP unless they are to be in house for over one month. Serology was performed by the supplier prior to shipment and on 4 sentinel animals after the study completion.
Serology was not performed at the same time as the final necropsies	None	The blood was drawn and frozen and then shipped to the vendor for the serology panel to be performed. All of this could not be done in one day
The sentinel animals were not randomly selected	None	The animals are assigned to the study based on body weights and the lighter animals were not used and some of these were used as sentinels
Animal # 45 (Rx code 71418) feed consumption was not weighed on day 14	Missing value, No effect on outcome of study	The feed for this animal was inadvertently not weighed on this day
Animal #219 (Rx code 59698) was dosed at 7:10 and sacrificed at 9:46 which is outside the approximately 2 hour window stated in the protocol, but still within the 7-10 AM 3 hour window for the sacrifice time period	None	There were too many animals to necropsy and still be within the approximately two hour window of dosing to necropsy.
Animal #161 (Rx code 18811) was necropsied at 10:51 which was outside the 7-10 necropsy window	None	There were too many animals to necropsy to have all of them fall within the 7-10 AM necropsy window on that day
The lot number of the linuron used was 301-91A whereas the protocol stated the lot number as 273-81B	None	It was assumed that the same lot number would be used in this study as a previous study and this was not done
An aliquot of each lot number of feed was suppose to be retained frozen for possible future analytical chemistry.	None	These samples were inadvertently not maintained. However, samples were saved from this lot of feed on another EDSP study.

Protocol Deviations		
Deviation	**Effect on Study**	**Reason**
Not all PM observations were initialed since they were performed by the same technician as the AM checks	None	Technicians did sign in on the room log and there was no designated space on the observation/dosing sheets for PM observations since they were added by the study director after significant lethargy was seen in the PM in some treated rats
The male rat weight range of initial weights at the start of the experiment was 362.5-450 grams whereas the protocol stated that the weight range for that age rats would be 326-350 grams	None	The age of the rats was correct and the males simply had a larger range of weights than expected when the protocol was written
All thyroids were not trimmed and weighed at EPL, Inc. The high dose and control group thyroids were trimmed and weighed at EPL, Inc., but the other dose groups were done at RTI at a later date.	This resulted in the weights of the control and high dose group thyroids being very similar, but those of the other dose groups were more dehydrated and weighed less as well as being trimmed by different people. This prevented the true statistical comparison from being performed across all dose groups and only the high dose and control groups could be accurately compared.	The histopathology was only to be done on the high dose group and the control group and so they were the only thyroids sent to EPL, Inc. Therefore they were the only groups processed by EPL, Inc. personnel.
The body weights for randomization were determined on Friday, May 16, 2003, prior to the start of dosing on Monday, May 19, 2003, instead of SD -1 (the day before experimental start) which would have been Sunday, May 18, 2003.	No apparent influence on the study. There was no significant difference in the initial SD 0 body weights between study dose groups.	This was done to allow for adequate time to complete the task.
Samples were only collected from the 2 middle dose groups of linuron (10 and 15 mg/mL) and methoxychlor (5.0 and 7.5 mg/mL) instead of from all dose groups	Did not affect the results of the study.	Technical staff misinterpreted the directions of the protocol.

Protocol Deviations		
Deviation	**Effect on Study**	**Reason**
Many of the samples taken for dose analysis of the inn-life formulations were less than 90% and greater than 110% of the target concentrations.	No apparent impact on the study.	Linuron and methoxychlor did not dissolve in methylcellulose and the suspensions were difficult to keep in suspension.

In the Study Director's professional opinion, these deviations did not affect the study integrity, performance, or interpretation, and are presented for completeness.

Carol D. Sloan
Study Director

4-25-05
Date

Table 2-A. Summary of the Fate of the Males Treated with Linuron (page 1 of 1)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
MALES	15	15	15	15	15
Fate of Animal					
Exposure Period	0	0	0	0	0
Scheduled Sacrifice on Study Day 14	15	15	15	15	15

Table 2-B. Summary of the Fate of the Males Treated with Methoxychlor (page 1 of 1)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
MALES	15	15	15	15	15
Fate of Animal					
Exposure Period	0	0	0	0	0
Scheduled Sacrifice on Study Day 14	15	15	15	15	15

Table 3-A. Summary and Statistical Analysis of the Linuron Treated Male Body Weights and Weight Changes During the Exposure Period
(page 1 of 2)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
No. of Males	15	15	15	15	15
Body Weight (sd 0) (g)[a]	401.2 ± 6.5 N=15	401.7 ± 5.8 N=15	402.9 ± 6.6 N=15	394.2 ± 5.2 N=15	405.1 ± 6.1 N=15
Body Weight (sd7) (g)[a]	439.6 ‡‡‡ ± 7.4 §§§ N=15	424.5 ± 5.6 N=15	406.5 ** ± 7.2 N=15	380.3 *** ± 5.8 N=15	391.0 *** ± 5.9 N=15
Body Weight (sd 14) (g)[a]	473.2 ‡‡‡ ± 8.6 §§§ N=15	447.2 * ± 7.8 N=15	424.7 *** ± 7.4 N=15	407.7 *** ± 6.6 N=15	414.8 *** ± 4.7 N=15
Body Weight Change (sd 0 to 7) (g)[a]	38.4 ‡‡‡ ± 3.0 §§§ N=15	22.8 ** ± 3.5 N=15	3.6 *** ± 3.2 N=15	-13.9 *** ± 2.0 N=15	-14.0 *** ± 5.0 N=15
Body Weight Change (sd 7 to 14) (g)[a]	33.6 ‡‡ ± 2.1	22.7 * ± 3.9 N=15	18.3 ** ± 2.9 N=15	27.4 ± 2.8 N=15	23.7 ± 2.1 N=15 N=15

(continued)

Table 3-A. Summary and Statistical Analysis of the Linuron Treated Male Body Weights and Weight Changes During the Exposure Period
(page 2 of 2)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Body Weight Change (sd 0 to 14) (g)[a]					
	72.0 ‡‡‡	45.5 ***	21.8 ***	13.5 ***	9.7 ***
	± 4.2 §§§	± 6.6	± 4.3	± 3.1	± 5.3
	N=15	N=15	N=15	N=15	N=15

[a]Reported as the mean ± S.E.M.; sd is study day with the first day of exposure being study day 0.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 3-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Body Weights and Weight Changes During the Exposure Period (page 1 of 2)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
No. of Males	15	15	15	15	15
Body Weight (sd 0) (g)[a]	401.2 ± 6.5 N=15	400.0 ± 5.9 N=15	393.6 ± 5.4 N=15	398.0 ± 5.7 N=15	396.9 ± 5.8 N=15
Body Weight (sd7) (g)[a]	439.6 ‡‡‡ ± 7.4 §§§ N=15	424.6 ± 6.0 N=15	412.0 ** ± 4.1 N=15	405.3 *** ± 5.5 N=15	398.1 *** ± 5.9 N=15
Body Weight (sd 14) (g)[a] #	473.2 ††† ± 8.6 ŸŸŸ N=15	447.6 Þ ± 6.0 N=15	434.3 ÞÞÞ ± 4.6 N=15	416.7 ÞÞÞ ± 6.2 N=15	417.1 ÞÞÞ ± 7.4 N=15
Body Weight Change (sd 0 to 7) (g)[a]	38.4 ‡‡‡ ± 3.0 §§§ N=15	24.5 ** ± 2.0 N=15	18.4 *** ± 2.0 N=15	7.3 *** ± 3.2 N=15	1.2 *** ± 3.2 N=15
Body Weight Change (sd 7 to 14) (g)[a] #	33.6 ††† ± 2.1 ŸŸŸ N=15	23.0 ÞÞÞ ± 1.7 N=15	22.3 ÞÞÞ ± 1.2 N=15	11.4 ÞÞÞ ± 1.8 N=15	19.0 ÞÞÞ ± 2.7 N=15

(continued)

Table 3-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Body Weights and Weight Changes During the Exposure Period (page 2 of 2)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Body Weight Change (sd 0 to 14) (g)[a]					
	72.0 ‡‡‡	47.5 ***	40.7 ***	18.8 ***	20.2 ***
	± 4.2 §§§	± 3.3	± 2.7	± 3.8	± 4.4
	N=15	N=15	N=15	N=15	N=15

[a]Reported as the mean ± S.E.M.; sd is study day with the first day of exposure being study day 0.
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡‡$p<0.001$; ANOVA Test.
§§§$p<0.001$; Test for Linear Trend.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.
Þ$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

Table 4-A. Summary and Statistical Analysis of the Linuron Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 1 of 3)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
No. of Males	15	15	15	15	15
Feed Consumption (sd 0 to 7) (g/day)[a]					
	30.6 ‡‡‡ ± 0.6 §§§ N=15	27.5 * ± 0.8 N=15	22.8 *** ± 0.8 N=15	20.5 *** ± 0.9 N=15	19.1 *** ± 0.8 N=15
Feed Consumption (sd 7 to 14) (g/day)[a]					
	29.5 ‡‡‡ ± 0.7 §§§ N=14[b]	27.5 ± 1.0 N=15	24.7 *** ± 0.8 N=15	25.8 ** ± 0.7 N=15	23.8 *** ± 0.4 N=14[c]
Feed Consumption (sd 0 to 14) (g/day)[a]					
	29.9 ‡‡‡ ± 0.7 §§§ N=14[d]	27.5 * ± 0.7 N=15	23.7 *** ± 0.7 N=15	23.1 *** ± 0.8 N=15	21.6 *** ± 0.6 N=14[d]
Feed Consumption (sd 0 to 7) (g/kg/day)[a]					
	72.9 ‡‡‡ ± 1.2 §§§ N=15	66.5 * ± 1.6 N=15	56.4 *** ± 1.7 N=15	52.7 *** ± 2.0 N=15	48.1 *** ± 2.0 N=15
Feed Consumption (sd 7 to 14) (g/kg/day)[a]					
	64.6 ‡‡ ± 1.1 N=14[b]	63.1 ± 2.0 N=15	59.3 * ± 1.6 N=15	65.4 ± 1.4 N=15	59.0 * ± 1.1 N=14[c]

(continued)

Table 4-A. Summary and Statistical Analysis of the Linuron Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 2 of 3)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Feed Consumption (sd 0 to 14) (g/kg/day)[a]					
	68.3 ‡‡‡ ± 1.0 §§§ N=14[d]	64.7 ± 1.3 N=15	57.7 *** ± 1.3 N=15	58.6 *** ± 1.5 N=15	53.3 *** ± 1.4 N=14[d]
Feed Efficiency (sd 0 to 7) (%)[a]					
	17.8 ‡‡‡ ± 1.2 §§§ N=15	11.7 ± 1.8 N=15	1.6 *** ± 2.0 N=15	-10.6 *** ± 1.9 N=15	-12.9 *** ± 5.0 N=15
Feed Efficiency (sd 7 to 14) (%)[a]					
	15.8 ‡ ± 0.8 N=14[b]	11.3 ± 1.8 N=15	10.2 * ± 1.5 N=15	15.2 ± 1.4 N=15	13.5 ± 1.1 N=14[c]
Feed Efficiency (sd 0 to 14) (%)[a]					
	16.6 ‡‡‡ ± 0.7 §§§ N=14[d]	11.5 * ± 1.6 N=15	6.3 *** ± 1.2 N=15	4.0 *** ± 0.9 N=15	2.5 *** ± 2.1 N=14[d]

(continued)

Table 4-A. Summary and Statistical Analysis of the Linuron Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 3 of 3)

[a]Reported as the mean ± S.E.M.; sd is study day with the first day of exposure being study day 0.
[b]Decrease in N is due to one male shredding his feed into the cage and therefore an accurate feed weight could not be obtained.
[c]Decrease in N is due to the feed weight for one male inadvertently not being weighed.
[d]Decrease in N is due to interim feed consumption value(s) for one or more males being missing and therefore the overall feed consumption value could not be calculated.
‡p<0.05; ANOVA Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 4-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 1 of 3)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
No. of Males	15	15	15	15	15
Feed Consumption (sd 0 to 7) (g/day)[a]					
	30.6 ‡‡‡ ± 0.6 §§§ N=15	27.4 ** ± 0.6 N=15	26.0 *** ± 0.6 N=15	25.1 *** ± 0.6 N=15	23.2 *** ± 0.8 N=15
Feed Consumption (sd 7 to 14) (g/day)[a]					
#	29.5 ††† ± 0.7 ŸŸŸ N=14[b]	26.1 ÞÞÞ ± 0.5 N=15	25.6 ÞÞÞ ± 0.4 N=15	22.1 ÞÞÞ ± 0.7 N=15	22.6 ÞÞÞ ± 1.2 N=15
Feed Consumption (sd 0 to 14) (g/day)[a]					
	29.9 ‡‡‡ ± 0.7 §§§ N=14[c]	26.7 ** ± 0.5 N=15	25.8 *** ± 0.4 N=15	23.6 *** ± 0.5 N=15	22.9 *** ± 0.9 N=15
Feed Consumption (sd 0 to 7) (g/kg/day)[a]					
	72.9 ‡‡‡ ± 1.2 §§§ N=15	66.4 ** ± 0.8 N=15	64.7 *** ± 1.7 N=15	62.5 *** ± 1.3 N=15	58.5 *** ± 1.8 N=15
Feed Consumption (sd 7 to 14) (g/kg/day)[a]					
#	64.6 ††† ± 1.1 ŸŸŸ N=14[b]	59.9 ÞÞÞ ± 0.7 N=15	60.5 ÞÞ ± 1.1 N=15	53.6 ÞÞÞ ± 1.0 N=15	55.2 ÞÞÞ ± 2.4 N=15

(continued)

Table 4-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 2 of 3)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Feed Consumption (sd 0 to 14) (g/kg/day)[a]					
#	68.3 ††† ± 1.0 ŸŸŸ N=14[c]	63.1 ÞÞÞ ± 0.7 N=15	62.5 ÞÞÞ ± 1.3 N=15	58.0 ÞÞÞ ± 0.9 N=15	56.6 ÞÞÞ ± 1.7 N=14[d]
Feed Efficiency (sd 0 to 7) (%)[a]					
	17.8 ‡‡‡ ± 1.2 §§§ N=15	12.7 ± 0.9 N=15	10.0 ** ± 1.0 N=15	3.9 *** ± 1.9 N=15	0.3 *** ± 2.0 N=15
Feed Efficiency (sd 7 to 14) (%)[a]					
	15.8 ‡‡‡ ± 0.8 §§§ N=14[b]	12.6 ± 0.9 N=15	12.5 ± 0.7 N=15	7.3 *** ± 1.1 N=15	11.4 ** ± 1.3 N=15
Feed Efficiency (sd 0 to 14) (%)[a]					
	16.6 ‡‡‡ ± 0.7 §§§ N=14[c]	12.7 * ± 0.8 N=15	11.3 *** ± 0.7 N=15	5.6 *** ± 1.1 N=15	5.9 *** ± 1.3 N=15

(continued)

Table 4-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Feed Consumption and Feed Efficiency During the Exposure Period (page 3 of 3)

[a]Reported as the mean ± S.E.M.; sd is study day with the first day of exposure being study day 0.
[b]Decrease in N is due to one male shredding his feed into the cage and therefore an accurate feed weight could not be obtained.
[c]Decrease in N is due to interim feed consumption value(s) for one or more males being missing and therefore the overall feed consumption value could not be calculated.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡‡$p<0.001$; ANOVA Test.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.
Þ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

Table 5-A. Summary of the Linuron Treated Male Clinical Observations During the Exposure Period (page 1 of 3)

A. Clinical Observations Summarized by Group

	Linuron (mg/kg/day, po)				
Observation	0	25	50	75	100
Ataxia and/or Ataxia, slight					1
Chromodacryorrhea					1
Efflux of the dosing solution			1	1	1
Heart: rapid beat					1
Lethargic and/or Lethargic, very					7
Piloerection	1		1	6	4
Rooting: post dosing					1
Rough coat					2
Salivation: prior to dosing					2
Sore(s)			1		
Teeth: malocclusion and upper right incisor chipped					1

B. Clinical Observations Summarized by Group and Day

		Linuron (mg/kg/day, po)				
Day[a]	Observation[b]	0	25	50	75	100
0	Efflux of the dosing solution			1		
1	Ataxia, slight					1
	Heart: rapid beat					1
	Lethargic, very					1
	Piloerection	1				

(continued)

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Linuron (mg/kg/day, po) 0	25	50	75	100
2	Ataxia					1
	Efflux of the dosing solution					1
	Lethargic					2
	Piloerection	1				1
3	Piloerection					1
	Rough coat					1
	Sore(s): head			1		
	Teeth: malocclusion and upper right incisor chipped[c]					1
4	Sore(s): head			1		
5	Rough coat					1
	Sore(s): head			1		
6	Piloerection				1	
	Rooting: post dosing					1
	Sore(s): head			1		
7	Efflux of the dosing solution				1	
	Piloerection				1	
	Sore(s): head			1		
8	Lethargic					1
	Piloerection				1	
	Salivation: prior to dosing					1
	Sore(s): head			1		

(continued)

Table 5-A. Summary of the Linuron Treated Male Clinical Observations During the Exposure Period (page 3 of 3)

B. Clinical Observations Summarized by Group and Day

		Linuron (mg/kg/day, po)				
Day[a]	Observation[b]	0	25	50	75	100
9	Lethargic					2
	Piloerection				3	
	Sore(s): head			1		
10	Piloerection					2
	Salivation: prior to dosing					1
	Sore(s): head			1		
11	Lethargic					1
	Piloerection					2
	Sore(s): head			1		
12	Lethargic					2
	Piloerection			1		
	Salivation: prior to dosing					1
	Sore(s): head			1		
13	Chromodacryorrhea: eye, right					1

[a]Study day.
[b]Clinical signs are tabulated once per day.
[c]No further notations were made unless a change occurred.

Table 5-B. Summary of the Methoxychlor Treated Male Clinical Observations During the Exposure Period (page 1 of 2)

A. Clinical Observations Summarized by Group

	Methoxychlor (mg/kg/day, po)				
Observation	0.0	12.5	25.0	37.5	50.0
Chromodacryorrhea					1
Efflux of the dosing solution					1
Feces: soft				1	
Piloerection	1	1	4	3	2
Rough coat					1
Rust colored fur					1

B. Clinical Observations Summarized by Group and Day

		Methoxychlor (mg/kg/day, po)				
Day[a]	Observation[b]	0.0	12.5	25.0	37.5	50.0
0	Chromodacryorrhea: eye, right					1
1	Chromodacryorrhea: eye, right					1
	Piloerection	1				
2	Chromodacryorrhea: eye, right, gone					1
	Piloerection	1		1		
4	Chromodacryorrhea: eye, right					1
5	Chromodacryorrhea: eye, right					1
6	Chromodacryorrhea: eye, right					1
	Piloerection			1		

(continued)

Table 5-B. Summary of the Methoxychlor Treated Male Clinical Observations During the Exposure Period (page 2 of 2)

B. Clinical Observations Summarized by Group and Day

		Methoxychlor (mg/kg/day, po)				
Day[a]	Observation[b]	0.0	12.5	25.0	37.5	50.0
7	Chromodacryorrhea: eye, right					1
	Feces: soft				1	
	Piloerection			1	1	1
	Rust colored fur: shoulder(s)					1
9	Chromodacryorrhea: eye, right					1
	Piloerection		1	1		
10	Chromodacryorrhea: eye, right					1
	Piloerection				2	1
11	Chromodacryorrhea: eye, right					1
	Efflux of the dosing solution					1
	Piloerection				2	
	Rough coat					1
12	Chromodacryorrhea: eye, right					1
13	Chromodacryorrhea: eye, right					1
14	Chromodacryorrhea: eye, right					1

[a]Study day.
[b]Clinical signs are tabulated once per day.

Table 6-A. Summary and Statistical Analysis of the Linuron Treated Male Sacrifice and Organ Weights (page 1 of 5)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
No. of Males	15	15	15	15	15
Sacrifice Body Weight (g)[a]					
	469.51 ‡‡‡ ± 8.63 §§§ N=15	444.54 ± 7.54 N=15	421.93 *** ± 7.26 N=15	405.67 *** ± 6.51 N=15	411.77 *** ± 4.91 N=15
Thyroid Weight (g)[a]					
	0.0275 ‡‡‡ ± 0.0013 N=15	0.0180 *** ± 0.0012 N=15	0.0169 *** ± 0.0007 N=15	0.0168 *** ± 0.0009 N=15	0.0278 ± 0.0013 N=15
Liver Weight (g)[a]					
	18.4040 ‡‡‡ ± 0.6095 §§§ N=15	17.0641 ± 0.5834 N=15	15.8888 ** ± 0.4582 N=15	15.1236 *** ± 0.3869 N=15	15.8994 ** ± 0.3141 N=15
Paired Testis Weight (g)[a]					
	3.3330 ± 0.0738 N=15	3.4221 ± 0.0538 N=15	3.2391 ± 0.1204 N=15	3.3098 ± 0.0409 N=15	3.2415 ± 0.1510 N=15
Paired Epididymis Weight (g)[a]					
	1.2044 ‡ ± 0.0231 §§ N=15	1.1949 ± 0.0208 N=15	1.1415 ± 0.0310 N=15	1.1276 ± 0.0248 N=15	1.0876 * ± 0.0446 N=15

(continued)

Table 6-A. Summary and Statistical Analysis of the Linuron Treated Male Sacrifice and Organ Weights (page 2 of 5)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Accessory Sex Gland Unit Weight (g)[a]					
	2.5291 ‡‡‡ ± 0.0716 §§§ N=15	2.4857 ± 0.0702 N=15	2.1126 ** ± 0.0673 N=15	2.1618 ** ± 0.1072 N=15	2.0721 *** ± 0.0727 N=15
Prostate Weight (g)[a]					
	0.9617 ‡‡ ± 0.0440 §§§ N=15	0.9308 ± 0.0303 N=15	0.8453 ± 0.0355 N=15	0.8175 * ± 0.0457 N=15	0.7733 ** ± 0.0365 N=15
Seminal Vesicles with Coagulating Glands Weight (g)[a]					
	1.5056 ‡‡ ± 0.0647 §§ N=15	1.4944 ± 0.0686 N=15	1.2028 ** ± 0.0554 N=15	1.2900 ± 0.0797 N=15	1.2531 * ± 0.0458 N=15
Relative Thyroid Weight (% of sacrifice weight)[a]					
	0.0059 ‡‡‡ ± 0.0003 N=15	0.0041 *** ± 0.0003 N=15	0.0040 *** ± 0.0002 N=15	0.0041 *** ± 0.0002 N=15	0.0067 ± 0.0003 N=15
Relative Liver Weight (% of sacrifice weight)[a]					
	3.9112 ± 0.0831 N=15	3.8264 ± 0.0809 N=15	3.7577 ± 0.0588 N=15	3.7260 ± 0.0694 N=15	3.8648 ± 0.0766 N=15

(continued)

Table 6-A. Summary and Statistical Analysis of the Linuron Treated Male Sacrifice and Organ Weights (page 3 of 5)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Relative Paired Testis Weight (% of sacrifice weight)[a]					
	0.7119 ‡	0.7723	0.7683	0.8184 **	0.7852
	± 0.0167 §	± 0.0158	± 0.0288	± 0.0152	± 0.0336
	N=15	N=15	N=15	N=15	N=15
Relative Paired Epididymis Weight (% of sacrifice weight)[a]					
	0.2573	0.2698	0.2706	0.2788	0.2637
	± 0.0055	± 0.0062	± 0.0065	± 0.0073	± 0.0100
	N=15	N=15	N=15	N=15	N=15
Relative Accessory Sex Gland Unit Weight (% of sacrifice weight)[a]					
	0.5398	0.5603	0.5018	0.5317	0.5032
	± 0.0148	± 0.0155	± 0.0165	± 0.0232	± 0.0163
	N=15	N=15	N=15	N=15	N=15
Relative Prostate Weight (% of sacrifice weight)[a]					
	0.2056	0.2106	0.2009	0.2012	0.1880
	± 0.0099	± 0.0084	± 0.0085	± 0.0107	± 0.0089
	N=15	N=15	N=15	N=15	N=15
Relative Seminal Vesicles with Coagulating Glands Weight (% of sacrifice weight)[a]					
	0.3212	0.3361	0.2857	0.3174	0.3041
	± 0.0129	± 0.0138	± 0.0138	± 0.0182	± 0.0100
	N=15	N=15	N=15	N=15	N=15
Thyroid Weight (g)[b]					
	0.0260 ƱƱƱ	0.0174 ???	0.0172 ???	0.0178 ???	0.0285
	± 0.0013 ƛ	± 0.0011	± 0.0011	± 0.0012	± 0.0011
	N=15	N=15	N=15	N=15	N=15

(continued)

Table 6-A. Summary and Statistical Analysis of the Linuron Treated Male Sacrifice and Organ Weights (page 4 of 5)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Liver Weight (g)[b]					
	16.3272 ± 0.3624 N=15	16.3232 ± 0.3113 N=15	16.3569 ± 0.3064 N=15	16.4615 ± 0.3291 N=15	16.9110 ± 0.3182 N=15
Paired Testis Weight (g)[b]					
	3.1689 ± 0.1115 N=15	3.3635 ± 0.0957 N=15	3.2761 ± 0.0942 N=15	3.4155 ± 0.1012 N=15	3.3215 ± 0.0979 N=15
Paired Epididymis Weight (g)[b]					
	1.1542 ± 0.0345 N=15	1.1769 ± 0.0297 N=15	1.1528 ± 0.0292 N=15	1.1600 ± 0.0313 N=15	1.1121 ± 0.0303 N=15
Accessory Sex Gland Unit Weight (g)[b]					
	2.3722 ± 0.0889 N=15	2.4297 ± 0.0764 N=15	2.1480 ± 0.0752 N=15	2.2630 ± 0.0807 N=15	2.1485 ± 0.0781 N=15
Prostate Weight (g)[b]					
	0.9322 ± 0.0463 N=15	0.9203 ± 0.0398 N=15	0.8520 ± 0.0391 N=15	0.8365 ± 0.0420 N=15	0.7877 ± 0.0406 N=15
Seminal Vesicles with Coagulating Glands Weight (g)[b]					
	1.3931 ± 0.0729 N=15	1.4543 ± 0.0626 N=15	1.2282 ± 0.0616 N=15	1.3625 ± 0.0662 N=15	1.3079 ± 0.0640 N=15

(continued)

Table 6-A. Summary and Statistical Analysis of the Linuron Treated Male Sacrifice and Organ Weights (page 5 of 5)

[a]Reported as the mean ± S.E.M.
[b]Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate).
‡p<0.05; ANOVA Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.
ʊʊʊp<0.001; Analysis of Covariance with body weight at sacrifice as the covariate.
ʎp≤0.05; Linear Trend Analysis of Covariance with body weight at sacrifice as the covariate.
ϟϟϟp<0.001; Dunnett's Test with body weight at sacrifice as the covariate.

Table 6-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Sacrifice and Organ Weights (page 1 of 5)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
No. of Males	15	15	15	15	15
Sacrifice Body Weight (g)[a]					
#	469.51 ††† ± 8.63 ŸŸŸ N=15	445.13 Þ ± 5.93 N=15	431.31 ÞÞÞ ± 4.61 N=15	414.87 ÞÞÞ ± 6.21 N=15	414.10 ÞÞÞ ± 7.51 N=15
Thyroid Weight (g)[a]					
	0.0275 ‡‡‡ ± 0.0013 N=15	0.0182 *** ± 0.0009 N=15	0.0174 *** ± 0.0008 N=15	0.0151 *** ± 0.0007 N=15	0.0282 ± 0.0011 N=15
Liver Weight (g)[a]					
	18.4040 ‡‡‡ ± 0.6095 §§§ N=15	16.3670 * ± 0.5234 N=15	16.5365 * ± 0.2949 N=15	15.6252 *** ± 0.3561 N=15	15.6550 *** ± 0.5276 N=15
Paired Testis Weight (g)[a]					
	3.3330 ± 0.0738 N=15	3.3100 ± 0.0821 N=15	3.2401 ± 0.0630 N=15	3.2638 ± 0.0698 N=15	3.3517 ± 0.0491 N=14[b]
Paired Epididymis Weight (g)[a]					
	1.2044 ‡ ± 0.0231 § N=15	1.1864 ± 0.0322 N=15	1.1579 ± 0.0219 N=15	1.0940 ** ± 0.0208 N=15	1.1513 ± 0.0188 N=15

(continued)

Table 6-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Sacrifice and Organ Weights
(page 2 of 5)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Accessory Sex Gland Unit Weight (g)[a]					
	2.5291 ‡‡‡	2.3920	2.1799 *	1.9956 ***	1.8866 ***
	± 0.0716 §§§	± 0.0696	± 0.0840	± 0.0853	± 0.1017
	N=15	N=15	N=15	N=15	N=15
Prostate Weight (g)[a]					
	0.9617 ‡‡	0.9415	0.8819	0.7830 *	0.7619 **
	± 0.0440 §§§	± 0.0287	± 0.0623	± 0.0405	± 0.0429
	N=15	N=15	N=15	N=15	N=15
Seminal Vesicles with Coagulating Glands Weight (g)[a]					
	1.5056 ‡‡‡	1.3872	1.2287 *	1.1634 **	1.0831 ***
	± 0.0647 §§§	± 0.0789	± 0.0711	± 0.0597	± 0.0772
	N=15	N=15	N=15	N=15	N=15
Relative Thyroid Weight (% of sacrifice weight)[a]					
#	0.0059 †††	0.0041 ÞÞÞ	0.0040 ÞÞÞ	0.0037 ÞÞÞ	0.0069 Þ
	± 0.0003	± 0.0002	± 0.0002	± 0.0002	± 0.0003
	N=15	N=15	N=15	N=15	N=15
Relative Liver Weight (% of sacrifice weight)[a]					
	3.9112	3.6662	3.8360	3.7642	3.7697
	± 0.0831	± 0.0755	± 0.0632	± 0.0560	± 0.0731
	N=15	N=15	N=15	N=15	N=15

(continued)

Table 6-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Sacrifice and Organ Weights
(page 3 of 5)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Relative Paired Testis Weight (% of sacrifice weight)[a]					
	0.7119 ‡‡ ± 0.0167 §§§ N=15	0.7469 ± 0.0242 N=15	0.7514 ± 0.0127 N=15	0.7889 * ± 0.0198 N=15	0.8207 *** ± 0.0213 N=14[b]
Relative Paired Epididymis Weight (% of sacrifice weight)[a]					
	0.2573 ± 0.0055 N=15	0.2675 ± 0.0087 N=15	0.2689 ± 0.0059 N=15	0.2643 ± 0.0054 N=15	0.2793 ± 0.0067 N=15
Relative Accessory Sex Gland Unit Weight (% of sacrifice weight)[a]					
	0.5398 ‡‡ ± 0.0148 §§§ N=15	0.5394 ± 0.0190 N=15	0.5061 ± 0.0199 N=15	0.4797 ± 0.0175 N=15	0.4535 ** ± 0.0205 N=15
Relative Prostate Weight (% of sacrifice weight)[a]					
#	0.2056 ± 0.0099 Ÿ N=15	0.2122 ± 0.0073 N=15	0.2055 ± 0.0149 N=15	0.1884 ± 0.0089 N=15	0.1835 ± 0.0091 N=15
Relative Seminal Vesicles with Coagulating Glands Weight (% of sacrifice weight)[a]					
	0.3212 ‡ ± 0.0129 §§ N=15	0.3131 ± 0.0193 N=15	0.2845 ± 0.0161 N=15	0.2797 ± 0.0129 N=15	0.2600 * ± 0.0167 N=15
Thyroid Weight (g)[c]					
	0.0278 ♦♦♦ ± 0.0014 N=15	0.0182 ◃◃◃ ± 0.0009 N=15	0.0174 ◃◃◃ ± 0.0007 N=15	0.0150 ◃◃◃ ± 0.0008 N=15	0.0281 ± 0.0013 N=15

(continued)

Table 6-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Sacrifice and Organ Weights
(page 4 of 5)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Liver Weight (g)[c]					
	16.4515 ♦	15.7932	16.7445	16.7624	16.8361
	± 0.3268	± 0.2558	± 0.3000	± 0.2345	± 0.2803
	N=15	N=15	N=15	N=15	N=15
Paired Testis Weight (g)[c]					
	3.3237	3.3071	3.2410	3.2691	3.3582
	± 0.0779	± 0.0807	± 0.0612	± 0.0672	± 0.0593
	N=15	N=15	N=15	N=15	N=14[b]
Paired Epididymis Weight (g)[c]					
	1.1983 ♦	1.1845	1.1586	1.0976 ◁◁	1.1551
	± 0.0239	± 0.0316	± 0.0214	± 0.0216	± 0.0204
	N=15	N=15	N=15	N=15	N=15
Accessory Sex Gland Unit Weight (g)[c]					
	2.3791 ♦	2.3479	2.1959	2.0830 ◁	1.9774 ◁◁
	± 0.0793 △△△	± 0.0804	± 0.0832	± 0.0722	± 0.0854
	N=15	N=15	N=15	N=15	N=15
Prostate Weight (g)[c]					
	0.9294 ♦	0.9320	0.8854	0.8018	0.7815 ◁
	± 0.0494 △△	± 0.0294	± 0.0628	± 0.0395	± 0.0417
	N=15	N=15	N=15	N=15	N=15
Seminal Vesicles with Coagulating Glands Weight (g)[c]					
	1.3998	1.3561	1.2400	1.2251	1.1471
	± 0.0649 △	± 0.0845	± 0.0647	± 0.0592	± 0.0686
	N=15	N=15	N=15	N=15	N=15

(continued)

Table 6-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Sacrifice and Organ Weights (page 5 of 5)

[a]Reported as the mean ± S.E.M.
[b]Decrease in N is due to one organ weight being a statistical outlier and therefore it was excluded.
[c]Reported as the adjusted mean ± S.E.M. (sacrifice weight as covariate).
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
Ÿ$p\leq0.05$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.
Þ$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
♦$p<0.05$; Wald Chi-square Test for overall treatment effect in robust regression model with body weight at sacrifice as covariate.
♦♦♦$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model with body weight at sacrifice as covariate.
△$p<0.05$; Linear trend test in robust regression model with body weight at sacrifice as covariate.
△△$p<0.01$; Linear trend test in robust regression model with body weight at sacrifice as covariate.
△△△$p<0.001$; Linear trend test in robust regression model with body weight at sacrifice as covariate.
◁$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model with body weight at sacrifice as covariate.
◁◁$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model with body weight at sacrifice as covariate.
◁◁◁$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model with body weight at sacrifice as covariate.

Table 7-A. Summary and Statistical Analysis of the Linuron Treated Male Hormone Data (page 1 of 2)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
No. of Males	15	15	15	15	15
Serum Testosterone (ng/ml)[a]					
	1.37 ± 0.17 N=15	1.97 ± 0.46 N=15	1.58 ± 0.34 N=15	0.87 ± 0.18 N=15	1.77 ± 0.43 N=15
Estradiol (pg/ml)[a]					
	40.84 ‡‡‡ ± 1.65 §§§ N=15	49.85 * ± 1.16 N=15	61.19 *** ± 2.46 N=15	63.74 *** ± 2.75 N=15	65.58 *** ± 2.94 N=15
Follicle Stimulating Hormone (ng/ml)[a]					
	16.19 ± 0.24 N=15	16.01 ± 0.42 N=15	17.17 ± 0.28 N=15	16.45 ± 0.45 N=15	16.11 ± 0.37 N=15
Luteinizing Hormone (ng/ml)[a]					
	3.22 ± 0.30 N=15	4.06 ± 0.48 N=15	3.96 ± 0.48 N=15	4.03 ± 0.46 N=15	3.19 ± 0.31 N=15
Thyroid Stimulating Hormone (ng/ml)[a]					
	12.33 ± 1.33 N=15	11.13 ± 0.79 N=15	10.59 ± 0.90 N=15	9.27 ± 0.45 N=15	10.61 ± 0.76 N=15
Thyroxine (ug/dL)[a]					
	4.18 ‡‡‡ ± 0.21 §§§ N=15	3.56 * ± 0.20 N=15	2.60 *** ± 0.15 N=15	2.52 *** ± 0.11 N=15	2.01 *** ± 0.13 N=15

(continued)

Table 7-A. Summary and Statistical Analysis of the Linuron Treated Male Hormone Data (page 2 of 2)

	Linuron (mg/kg/day, po)				
	0	25	50	75	100
Triiodothyronine (ng/dL)[a]					
	78.41 ± 5.09 N=15	83.89 ± 5.51 N=15	70.91 ± 4.10 N=15	75.37 ± 3.77 N=15	79.34 ± 4.41 N=15
Prolactin (ng/ml)[a]					
	60.15 ‡ ± 8.10 §§ N=15	48.88 ± 5.66 N=15	42.56 ± 4.40 N=15	47.17 ± 4.91 N=15	32.86 ** ± 4.27 N=15
Dihydrotestosterone (pg/ml)[a]					
	77.82 ± 8.82 N=15	108.13 ± 19.14 N=15	97.01 ± 14.61 N=15	64.49 ± 5.69 N=15	105.45 ± 16.91 N=15

[a]Reported as the mean ± S.E.M.
‡p<0.05; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 7-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Hormone Data (page 1 of 2)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
No. of Males	15	15	15	15	15
Serum Testosterone (ng/ml)[a]					
	1.37 ± 0.17 N=15	1.86 ± 0.53 N=15	1.75 ± 0.38 N=15	1.07 ± 0.24 N=15	1.02 ± 0.23 N=15
Estradiol (pg/ml)[a]					
	40.84 ± 1.65 § N=15	42.21 ± 2.06 N=15	44.93 ± 1.78 N=15	46.46 ± 2.38 N=15	47.44 ± 2.68 N=15
Follicle Stimulating Hormone (ng/ml)[a]					
	16.19 ± 0.24 N=15	16.45 ± 0.39 N=15	16.25 ± 0.29 N=15	16.14 ± 0.35 N=15	16.14 ± 0.35 N=15
Luteinizing Hormone (ng/ml)[a]					
	3.22 ± 0.30 N=15	3.35 ± 0.23 N=15	3.16 ± 0.17 N=15	3.78 ± 0.29 N=15	3.63 ± 0.34 N=15
Thyroid Stimulating Hormone (ng/ml)[a]					
	12.33 ± 1.33 N=15	10.81 ± 0.94 N=15	12.79 ± 0.86 N=15	11.54 ± 0.80 N=15	12.65 ± 1.46 N=15
Thyroxine (ug/dL)[a]					
	4.18 ‡ ± 0.21 § N=15	4.46 ± 0.17 N=15	5.02 * ± 0.22 N=15	4.51 ± 0.17 N=15	4.80 ± 0.21 N=15

(continued)

Table 7-B. Summary and Statistical Analysis of the Methoxychlor Treated Male Hormone Data (page 2 of 2)

	Methoxychlor (mg/kg/day, po)				
	0.0	12.5	25.0	37.5	50.0
Triiodothyronine (ng/dL)[a]					
	78.41 ± 5.09 N=15	78.41 ± 4.83 N=15	84.15 ± 3.49 N=15	79.57 ± 4.59 N=15	75.76 ± 3.33 N=15
Prolactin (ng/ml)[a]					
	60.15 ‡‡‡ ± 8.10 §§§ N=15	53.41 ± 8.78 N=15	118.71 * ± 16.31 N=15	95.12 ± 19.05 N=15	154.47 *** ± 16.04 N=15
Dihydrotestosterone (pg/ml)[a]					
	77.82 ± 8.82 N=15	97.46 ± 17.25 N=15	100.26 ± 17.11 N=15	71.24 ± 10.86 N=15	69.78 ± 8.60 N=15

[a]Reported as the mean ± S.E.M.
‡p<0.05; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 8-A. Summary of the Linuron Treated Male Gross Necropsy Findings (page 1 of 1)

Gross Necropsy Findings

	Linuron (mg/kg/day, po)				
Finding	0	25	50	75	100
Epididymis: reduced in size, bilateral			1		1
Prostate: reduced in size				1	
Seminal Vesicles: reduced in size				1	
Testis: reduced in size, bilateral			1		2

Table 8-B. Summary of the Methoxychlor Treated Male Gross Necropsy Findings (page 1 of 1)

Gross Necropsy Findings

	Methoxychlor (mg/kg/day, po)				
Finding	0.0	12.5	25.0	37.5	50.0
Prostate: reduced in size					2
Seminal Vesicles: reduced in size					2

Table A-1. Individual Male Fate (page 1 of 4)

Group[a]	Male ID	Study Phase	Study Day[b]
1	1	Scheduled Sacrifice	14
	35	Scheduled Sacrifice	14
	37	Scheduled Sacrifice	14
	71	Scheduled Sacrifice	14
	73	Scheduled Sacrifice	14
	107	Scheduled Sacrifice	14
	109	Scheduled Sacrifice	14
	143	Scheduled Sacrifice	14
	145	Scheduled Sacrifice	14
	179	Scheduled Sacrifice	14
	181	Scheduled Sacrifice	14
	215	Scheduled Sacrifice	14
	217	Scheduled Sacrifice	14
	251	Scheduled Sacrifice	14
	253	Scheduled Sacrifice	14
2	3	Scheduled Sacrifice	14
	33	Scheduled Sacrifice	14
	39	Scheduled Sacrifice	14
	69	Scheduled Sacrifice	14
	75	Scheduled Sacrifice	14
	105	Scheduled Sacrifice	14
	111	Scheduled Sacrifice	14
	141	Scheduled Sacrifice	14
	147	Scheduled Sacrifice	14
	177	Scheduled Sacrifice	14
	183	Scheduled Sacrifice	14
	213	Scheduled Sacrifice	14
	219	Scheduled Sacrifice	14
	249	Scheduled Sacrifice	14
	255	Scheduled Sacrifice	14
3	5	Scheduled Sacrifice	14
	31	Scheduled Sacrifice	14
	41	Scheduled Sacrifice	14
	67	Scheduled Sacrifice	14
	77	Scheduled Sacrifice	14
	103	Scheduled Sacrifice	14
	113	Scheduled Sacrifice	14

(continued)

Table A-1. Individual Male Fate (page 2 of 4)

Group[a]	Male ID	Study Phase	Study Day[b]
3	139	Scheduled Sacrifice	14
	149	Scheduled Sacrifice	14
	175	Scheduled Sacrifice	14
	185	Scheduled Sacrifice	14
	211	Scheduled Sacrifice	14
	221	Scheduled Sacrifice	14
	247	Scheduled Sacrifice	14
	257	Scheduled Sacrifice	14
4	7	Scheduled Sacrifice	14
	29	Scheduled Sacrifice	14
	43	Scheduled Sacrifice	14
	65	Scheduled Sacrifice	14
	79	Scheduled Sacrifice	14
	101	Scheduled Sacrifice	14
	115	Scheduled Sacrifice	14
	137	Scheduled Sacrifice	14
	151	Scheduled Sacrifice	14
	173	Scheduled Sacrifice	14
	187	Scheduled Sacrifice	14
	209	Scheduled Sacrifice	14
	223	Scheduled Sacrifice	14
	245	Scheduled Sacrifice	14
	259	Scheduled Sacrifice	14
5	9	Scheduled Sacrifice	14
	27	Scheduled Sacrifice	14
	45	Scheduled Sacrifice	14
	63	Scheduled Sacrifice	14
	81	Scheduled Sacrifice	14
	99	Scheduled Sacrifice	14
	117	Scheduled Sacrifice	14
	135	Scheduled Sacrifice	14
	153	Scheduled Sacrifice	14
	171	Scheduled Sacrifice	14
	189	Scheduled Sacrifice	14
	207	Scheduled Sacrifice	14
	225	Scheduled Sacrifice	14
	243	Scheduled Sacrifice	14
	261	Scheduled Sacrifice	14

(continued)

Table A-1. Individual Male Fate (page 3 of 4)

Group[a]	Male ID	Study Phase	Study Day[b]
6	11	Scheduled Sacrifice	14
	25	Scheduled Sacrifice	14
	47	Scheduled Sacrifice	14
	61	Scheduled Sacrifice	14
	83	Scheduled Sacrifice	14
	97	Scheduled Sacrifice	14
	119	Scheduled Sacrifice	14
	133	Scheduled Sacrifice	14
	155	Scheduled Sacrifice	14
	169	Scheduled Sacrifice	14
	191	Scheduled Sacrifice	14
	205	Scheduled Sacrifice	14
	227	Scheduled Sacrifice	14
	241	Scheduled Sacrifice	14
	263	Scheduled Sacrifice	14
7	13	Scheduled Sacrifice	14
	23	Scheduled Sacrifice	14
	49	Scheduled Sacrifice	14
	59	Scheduled Sacrifice	14
	85	Scheduled Sacrifice	14
	95	Scheduled Sacrifice	14
	121	Scheduled Sacrifice	14
	131	Scheduled Sacrifice	14
	157	Scheduled Sacrifice	14
	167	Scheduled Sacrifice	14
	193	Scheduled Sacrifice	14
	203	Scheduled Sacrifice	14
	229	Scheduled Sacrifice	14
	239	Scheduled Sacrifice	14
	265	Scheduled Sacrifice	14
8	15	Scheduled Sacrifice	14
	21	Scheduled Sacrifice	14
	51	Scheduled Sacrifice	14
	57	Scheduled Sacrifice	14
	87	Scheduled Sacrifice	14
	93	Scheduled Sacrifice	14
	123	Scheduled Sacrifice	14

(continued)

Table A-1. Individual Male Fate (page 4 of 4)

Group[a]	Male ID	Study Phase	Study Day[b]
8	129	Scheduled Sacrifice	14
	159	Scheduled Sacrifice	14
	165	Scheduled Sacrifice	14
	195	Scheduled Sacrifice	14
	201	Scheduled Sacrifice	14
	231	Scheduled Sacrifice	14
	237	Scheduled Sacrifice	14
	267	Scheduled Sacrifice	14
9	17	Scheduled Sacrifice	14
	19	Scheduled Sacrifice	14
	53	Scheduled Sacrifice	14
	55	Scheduled Sacrifice	14
	89	Scheduled Sacrifice	14
	91	Scheduled Sacrifice	14
	125	Scheduled Sacrifice	14
	127	Scheduled Sacrifice	14
	161	Scheduled Sacrifice	14
	163	Scheduled Sacrifice	14
	197	Scheduled Sacrifice	14
	199	Scheduled Sacrifice	14
	233	Scheduled Sacrifice	14
	235	Scheduled Sacrifice	14
	269	Scheduled Sacrifice	14

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.
[b]Study day 0 was the first day of exposure.

Table A-2. Individual Male Body Weights (g) During the Exposure Period (page 1 of 4)

Group[a]	Male ID	Study Day 0	Study Day 7	Study Day 14
1	1	363.7	403.4	433.1
	35	368.3	401.7	421.0
	37	378.8	437.2	483.3
	71	380.4	414.3	437.0
	73	385.1	416.1	444.5
	107	382.6	409.2	439.1
	109	401.3	456.7	496.6
	143	394.8	431.8	467.2
	145	405.8	456.4	488.2
	179	404.9	418.5	453.1
	181	422.4	453.4	500.3
	215	422.7	469.1	506.2
	217	420.0	455.7	490.3
	251	446.0	489.6	532.3
	253	441.2	481.0	506.0
2	3	371.2	405.2	457.8
	33	383.6	425.6	471.5
	39	375.6	402.5	422.1
	69	383.7	404.3	417.6
	75	378.2	401.0	413.7
	105	390.5	408.6	430.5
	111	406.7	441.8	470.2
	141	397.8	425.5	437.0
	147	406.5	447.3	467.4
	177	407.3	432.1	441.6
	183	416.7	437.4	471.7
	213	405.5	399.1	394.3
	219	412.6	416.7	431.7
	249	445.7	471.1	509.1
	255	444.2	450.0	472.5
3	5	367.5	369.5	385.4
	31	369.7	361.8	385.3
	41	377.7	383.9	415.9
	67	385.8	397.4	440.4
	77	379.9	388.8	399.5
	103	390.6	404.0	422.8
	113	394.9	381.0	392.8

(continued)

Table A-2. Individual Male Body Weights (g) During the Exposure Period (page 2 of 4)

Group[a]	Male ID	Study Day 0	Study Day 7	Study Day 14
3	139	417.5	442.2	456.0
	149	404.9	425.1	439.8
	175	407.6	406.6	427.5
	185	407.9	406.0	408.5
	211	411.9	406.9	407.1
	221	438.3	421.3	455.1
	247	439.3	454.9	470.4
	257	450.0	447.7	464.5
4	7	365.6	352.2	395.2
	29	371.3	348.3	370.9
	43	369.4	359.9	380.4
	65	386.3	381.4	424.7
	79	393.0	388.9	413.5
	101	377.5	348.8	377.4
	115	396.9	377.7	405.5
	137	384.9	366.7	379.6
	151	394.8	373.6	407.2
	173	392.3	387.4	406.4
	187	407.7	400.5	411.4
	209	396.4	389.7	404.4
	223	421.2	403.7	434.9
	245	422.5	402.8	442.6
	259	433.9	423.1	462.0
5	9	371.3	353.7	390.3
	27	376.4	361.1	391.0
	45	383.1	360.2	403.0
	63	380.0	367.0	388.5
	81	386.2	391.1	416.0
	99	400.3	404.6	420.8
	117	399.1	394.8	425.5
	135	411.1	403.2	424.3
	153	398.9	396.8	417.9
	171	411.5	408.7	430.0
	189	411.7	402.0	416.2
	207	428.4	409.6	433.5
	225	436.2	437.2	452.2
	243	432.2	397.8	416.1
	261	449.6	377.8	396.2

(continued)

Table A-2. Individual Male Body Weights (g) During the Exposure Period (page 3 of 4)

Group[a]	Male ID	Study Day 0	Study Day 7	Study Day 14
6	11	369.5	392.0	412.1
	25	365.5	386.6	413.6
	47	376.3	404.7	430.2
	61	381.9	404.2	419.7
	83	387.3	416.3	449.6
	97	388.1	422.8	450.1
	119	395.6	423.6	445.3
	133	401.5	424.9	441.0
	155	409.9	418.7	438.8
	169	404.9	424.2	450.1
	191	414.2	444.7	473.9
	205	416.1	454.6	485.7
	227	415.2	441.0	466.7
	241	424.9	438.0	451.4
	263	449.4	472.1	485.3
7	13	362.5	389.1	414.2
	23	371.0	390.6	413.7
	49	366.7	395.2	418.4
	59	378.2	396.4	419.1
	85	384.5	412.1	440.9
	95	375.4	394.2	407.4
	121	391.5	401.7	415.8
	131	400.1	422.2	446.4
	157	393.1	415.8	434.4
	167	404.1	420.6	449.3
	193	405.9	426.4	448.5
	203	406.2	429.2	455.1
	229	407.0	421.5	443.8
	239	424.5	427.6	445.8
	265	433.6	437.4	461.3
8	15	369.9	375.4	383.1
	21	370.9	379.9	388.9
	51	374.2	375.5	377.6
	57	381.1	401.8	412.6
	87	376.7	403.1	417.2
	93	384.6	407.0	420.5
	123	396.3	397.4	424.0

(continued)

Table A-2. Individual Male Body Weights (g) During the Exposure Period (page 4 of 4)

Group[a]	Male ID	Study Day 0	Study Day 7	Study Day 14
8	129	401.4	382.1	388.6
	159	390.0	399.0	409.0
	165	404.0	416.9	427.0
	195	417.3	426.3	424.6
	201	414.2	431.2	447.9
	231	428.8	417.4	429.6
	237	421.6	419.2	439.0
	267	438.4	447.0	461.1
9	17	363.3	365.9	377.6
	19	368.0	376.2	383.0
	53	373.8	376.0	392.9
	55	394.0	371.1	390.6
	89	384.0	374.8	376.7
	91	388.1	406.1	442.4
	125	382.2	396.0	425.8
	127	397.4	387.3	405.0
	161	396.6	394.4	421.3
	163	406.7	420.7	439.6
	197	395.5	415.4	427.0
	199	419.7	414.6	445.4
	233	410.9	406.7	411.4
	235	437.5	425.1	450.6
	269	436.5	441.2	467.8

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.

Table A-3. Individual Male Feed Consumption (g/day) During the Exposure Period (page 1 of 4)

Group[a]	Male ID	Study Days 0-7	7-14	0-14
1	1	26.3	24.3	25.3
	35	27.9	25.7	26.8
	37	34.5	.[b]	.[d]
	71	27.6	26.6	27.1
	73	31.0	29.7	30.3
	107	28.6	29.0	28.8
	109	30.6	30.3	30.4
	143	32.4	33.4	32.9
	145	32.2	30.5	31.4
	179	27.5	26.5	27.0
	181	32.2	31.8	32.0
	215	33.8	32.4	33.1
	217	31.5	30.4	31.0
	251	32.1	31.8	31.9
	253	31.3	30.6	30.9
2	3	25.0	28.8	26.9
	33	27.0	38.9	33.0
	39	23.0	28.5	25.7
	69	25.9	24.4	25.1
	75	26.9	23.1	25.0
	105	26.2	26.2	26.2
	111	31.0	27.3	29.1
	141	29.7	28.2	28.9
	147	29.2	26.4	27.8
	177	33.9	24.9	29.4
	183	26.8	27.3	27.1
	213	23.6	23.6	23.6
	219	27.6	27.5	27.5
	249	30.8	32.7	31.7
	255	25.5	25.0	25.2
3	5	20.8	21.3	21.1
	31	17.7	22.9	20.3
	41	23.2	26.3	24.7
	67	24.1	28.3	26.2
	77	26.3	24.9	25.6
	103	22.9	26.3	24.6
	113	15.8	22.7	19.3

(continued)

Table A-3. Individual Male Feed Consumption (g/day) During the Exposure Period (page 2 of 4)

Group[a]	Male ID	Study Days 0-7	7-14	0-14
3	139	27.8	27.6	27.7
	149	25.1	26.4	25.8
	175	21.2	21.9	21.5
	185	23.7	19.5	21.6
	211	22.5	19.4	21.0
	221	23.8	29.1	26.5
	247	24.2	25.8	25.0
	257	23.3	27.3	25.3
4	7	19.9	24.3	22.1
	29	13.1	20.8	17.0
	43	19.7	21.8	20.7
	65	24.2	30.4	27.3
	79	24.0	25.9	25.0
	101	16.4	24.5	20.5
	115	20.0	25.3	22.7
	137	19.8	25.2	22.5
	151	20.2	24.5	22.3
	173	19.3	28.1	23.7
	187	25.8	29.0	27.4
	209	19.0	24.1	21.5
	223	17.6	24.8	21.2
	245	23.1	30.2	26.7
	259	24.7	28.3	26.5
5	9	16.9	24.3	20.6
	27	16.0	23.3	19.7
	45	16.2	.[c]	.[d]
	63	19.2	23.4	21.3
	81	22.0	24.8	23.4
	99	22.1	23.6	22.8
	117	19.2	21.9	20.5
	135	21.0	22.9	22.0
	153	22.4	27.0	24.7
	171	22.8	23.6	23.2
	189	22.0	24.4	23.2
	207	15.7	23.2	19.5
	225	23.2	26.9	25.1
	243	14.6	22.7	18.7
	261	14.0	21.5	17.7

(continued)

Table A-3. Individual Male Feed Consumption (g/day) During the Exposure Period (page 3 of 4)

Group[a]	Male ID	Study Days 0-7	7-14	0-14
6	11	25.3	23.8	24.6
	25	25.7	25.4	25.5
	47	26.0	25.3	25.7
	61	24.8	22.5	23.6
	83	27.9	27.2	27.5
	97	28.6	27.0	27.8
	119	26.9	25.5	26.2
	133	26.6	25.1	25.9
	155	26.0	27.1	26.5
	169	25.8	24.3	25.0
	191	27.6	26.8	27.2
	205	31.6	29.3	30.4
	227	28.0	26.2	27.1
	241	27.7	26.8	27.2
	263	32.2	29.7	31.0
7	13	30.0	28.7	29.4
	23	24.3	23.0	23.6
	49	25.0	24.1	24.5
	59	24.5	23.7	24.1
	85	26.9	26.5	26.7
	95	23.9	26.5	25.2
	121	26.7	25.4	26.1
	131	27.0	24.8	25.9
	157	29.9	26.3	28.1
	167	26.4	27.6	27.0
	193	28.0	25.4	26.7
	203	26.9	25.7	26.3
	229	22.3	23.1	22.7
	239	24.8	25.7	25.2
	265	23.3	26.8	25.1
8	15	23.4	19.8	21.6
	21	22.5	19.7	21.1
	51	26.2	20.2	23.2
	57	23.0	19.8	21.4
	87	27.0	25.0	26.0
	93	27.9	22.7	25.3
	123	22.1	21.8	22.0

(continued)

Table A-3. Individual Male Feed Consumption (g/day) During the Exposure Period (page 4 of 4)

Group[a]	Male ID	Study Days 0-7	7-14	0-14
8	129	20.8	19.8	20.3
	159	25.5	19.5	22.5
	165	26.6	22.0	24.3
	195	26.1	22.2	24.1
	201	25.8	25.8	25.8
	231	27.7	21.1	24.4
	237	24.7	23.9	24.3
	267	27.2	27.9	27.5
9	17	20.0	18.5	19.2
	19	25.0	15.5	20.2
	53	23.5	19.2	21.3
	55	19.5	21.9	20.7
	89	21.6	16.8	19.2
	91	28.2	27.3	27.8
	125	24.4	24.3	24.4
	127	23.6	22.1	22.9
	161	23.3	29.0	26.1
	163	24.8	23.3	24.0
	197	21.3	20.4	20.8
	199	22.3	26.1	24.2
	233	19.5	17.9	18.7
	235	21.6	24.3	22.9
	269	30.3	32.6	31.5

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.
[b]Feed consumption value was excluded because the male had shredded feed into the cage and an accurate feed weight could not be obtained.
[c]Feed weight was inadvertently not weighed.
[d]Interim feed consumption value(s) are missing and therefore this overall value could not be calculated.

Table A-4. Individual Male Clinical Observations During the Exposure Period (page 1 of 3)

Group[a]	Male ID	Study Day	Clinical Observation
1	253	1	Piloerection
		2	Piloerection
3	77	0	Efflux of the dosing solution
	113	12	Piloerection
	211	3	Sore(s): head
		4	Sore(s): head
		5	Sore(s): head
		6	Sore(s): head
		7	Sore(s): head
		8	Sore(s): head
		9	Sore(s): head
		10	Sore(s): head
		11	Sore(s): head
		12	Sore(s): head
4	101	9	Piloerection
	137	7	Efflux of the dosing solution
		8	Piloerection
	187	9	Piloerection
	209	7	Piloerection
	245	6	Piloerection
	259	9	Piloerection
5	9	10	Piloerection
		11	Piloerection
			Lethargic
	27	2	Lethargic
		3	Piloerection
		5	Rough coat
		10	Piloerection
	99	1	Lethargic, very
			Heart: rapid beat
		2	Piloerection
		8	Salivation: prior to dosing
		10	Salivation: prior to dosing
	117	2	Efflux of the dosing solution
		3	Teeth: malocclusion and upper right incisor chipped[b]

(continued)

Table A-4. Individual Male Clinical Observations During the Exposure Period (page 2 of 3)

Group[a]	Male ID	Study Day	Clinical Observation
5	135	9	Lethargic
		12	Lethargic
	153	3	Rough coat
		6	Rooting: post dosing
		9	Lethargic
		12	Lethargic
		13	Chromodacryorrhea: eye, right
	171	11	Piloerection
	189	8	Lethargic
	207	1	Ataxia, slight
		2	Ataxia
			Lethargic
		12	Salivation: prior to dosing
6	83	9	Piloerection
7	59	2	Piloerection
	85	9	Piloerection
	167	7	Piloerection
	239	6	Piloerection
8	21	10	Piloerection
		11	Piloerection
	51	7	Feces: soft
		10	Piloerection
		11	Piloerection
	201	7	Piloerection
9	19	11	Rough coat
	53	10	Piloerection
	197	7	Piloerection
	199	7	Rust colored fur: shoulder(s)
	269	0	Chromodacryorrhea: eye, right
		1	Chromodacryorrhea: eye, right
		2	Chromodacryorrhea: eye, right, gone
		4	Chromodacryorrhea: eye, right
		5	Chromodacryorrhea: eye, right

(continued)

Table A-4. Individual Male Clinical Observations During the Exposure Period (page 3 of 3)

Group[a]	Male ID	Study Day	Clinical Observation
9	269	6	Chromodacryorrhea: eye, right
		7	Chromodacryorrhea: eye, right
		9	Chromodacryorrhea: eye, right
		10	Chromodacryorrhea: eye, right
		11	Chromodacryorrhea: eye, right
			Efflux of the dosing solution
		12	Chromodacryorrhea: eye, right
		13	Chromodacryorrhea: eye, right
		14	Chromodacryorrhea: eye, right

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.
[b]No further notation made unless a change occurred.

Table A-5. Individual Male Sacrifice and Organ Weights (g) (page 1 of 4)

Group[a]	Male ID	Sacrifice Weight	Thyroid	Liver	Paired Testis	Paired Epididymis	Accesory Sex Gland Unit	Prostate	Seminal Vesicles with Coagulating Glands
1	1	430.80	0.0288	15.7835	3.1767	1.1660	2.4528	0.9048	1.4933
	35	417.46	0.0330	15.0030	3.3259	1.2083	2.4432	1.0208	1.4083
	37	481.80	0.0279	20.0811	2.9526	1.1736	2.3362	0.8067	1.4718
	71	431.23	0.0202	15.0647	2.9073	1.1395	2.7852	1.2234	1.5481
	73	440.25	0.0262	18.2904	3.5060	1.1119	2.3530	0.6939	1.6022
	107	435.77	0.0311	16.0793	3.4213	1.2418	2.4737	1.0053	1.3711
	109	492.46	0.0295	18.2002	3.3613	1.1560	2.4666	0.7803	1.6328
	143	464.77	0.0221	19.3255	3.0768	1.1213	1.9765	0.7163	1.0924
	145	486.19	0.0185	18.0945	3.8984	1.3592	2.2807	1.1020	1.1304
	179	448.10	0.0326	17.4729	3.1026	1.0817	2.4199	0.9829	1.4078
	181	491.56	0.0342	21.0710	3.2579	1.2415	2.6773	1.1285	1.5095
	215	502.10	0.0311	23.5816	3.1923	1.2895	3.1416	0.8598	2.1597
	217	486.92	0.0222	18.7029	3.8088	1.3904	2.6557	0.8949	1.7281
	251	527.68	0.0295	20.4308	3.5565	1.1485	2.8875	1.1996	1.6240
	253	505.63	0.0257	18.8780	3.4513	1.2375	2.5873	1.1060	1.4051
2	3	454.55	0.0136	17.6458	3.2737	1.0826	2.1299	0.8226	1.2837
	33	468.85	0.0190	18.6586	3.7316	1.2171	2.1123	0.8397	1.1857
	39	420.41	0.0167	17.2747	3.3388	1.0908	2.1489	0.7588	1.3507
	69	414.72	0.0165	15.9513	2.9891	1.0399	2.3722	1.0613	1.2506
	75	411.60	0.0124	14.7770	3.2072	1.1867	2.4867	1.1533	1.2862
	105	430.01	0.0267	16.9440	3.5837	1.2556	2.8328	0.9484	1.7738
	111	463.20	0.0111	19.2085	3.4576	1.2196	2.4306	1.1446	1.2327
	141	429.45	0.0216	16.4086	3.7470	1.2791	2.6011	0.9987	1.5572
	147	463.74	0.0197	19.4235	3.2685	1.2084	2.9160	0.8476	1.9975
	177	439.11	0.0187	14.8741	3.5212	1.2943	2.3267	0.9140	1.3689
	183	462.52	0.0264	17.5611	3.5583	1.1977	2.9177	0.9168	1.9189
	213	398.30	0.0174	12.3701	3.3273	1.1829	2.2827	0.8415	1.3870
	219	431.29	0.0212	15.1255	3.6335	1.2586	2.4887	0.9143	1.5022
	249	509.15	0.0160	21.4359	3.3493	1.1126	2.7445	0.8355	1.8316
	255	471.17	0.0126	18.3030	3.3448	1.2972	2.4953	0.9656	1.4898
3	5	382.84	0.0157	13.5768	3.2268	1.0754	1.5911	0.8766	0.6833
	31	381.81	0.0166	13.9440	1.6708	0.7486	2.0075	0.7595	1.2141
	41	415.89	0.0137	16.3592	3.1495	1.1046	2.0047	0.7093	1.2369
	67	433.20	0.0173	18.4739	3.4553	1.2451	2.3761	0.8684	1.3996
	77	398.26	0.0207	14.7334	3.4072	1.1882	2.6940	1.0360	1.5338
	103	421.44	0.0148	16.2943	3.6739	1.1947	2.2195	0.8293	1.3786
	113	391.02	0.0138	13.2953	3.3266	1.1090	1.9338	0.7452	1.1302
	139	453.49	0.0231	18.2831	3.1407	1.1718	2.3222	1.1100	1.1368
	149	434.92	0.0170	16.5470	3.1210	1.1565	2.0128	0.8790	1.0030
	175	421.83	0.0175	15.8763	3.5602	1.2295	2.0344	0.8762	1.1357
	185	405.42	0.0172	14.0011	3.5381	1.1817	2.0902	0.6210	1.4319
	211	407.95	0.0146	14.4755	3.2172	1.1234	2.0131	0.9376	1.0272
	221	448.44	0.0184	17.2961	3.4505	1.2480	2.4090	0.9396	1.4065
	247	466.51	0.0138	18.0025	3.2079	1.1545	2.0829	0.8718	1.0814
	257	465.98	0.0194	17.1732	3.4405	1.1913	1.8978	0.6200	1.2428

(continued)

Table A-5. Individual Male Sacrifice and Organ Weights (g) (page 2 of 4)

Group[a]	Male ID	Sacrifice Weight	Thyroid	Liver	Paired Testis	Paired Epididymis	Accesory Sex Gland Unit	Prostate	Seminal Vesicles with Coagulating Glands
4	7	386.46	0.0145	16.0586	3.0056	0.9509	2.1224	0.7345	1.3472
	29	369.79	0.0128	13.1792	3.4086	1.1352	1.8299	0.6436	1.1615
	43	381.59	0.0153	14.8431	3.2382	1.0381	1.9388	0.6658	1.2457
	65	423.27	0.0184	15.5058	3.3816	1.2117	2.3424	0.8724	1.3171
	79	409.45	0.0159	15.9336	3.0124	1.1008	2.4597	0.8379	1.5760
	101	377.73	0.0139	12.5362	3.4619	1.2542	2.4973	1.2356	1.2374
	115	406.62	0.0148	15.2658	3.2289	1.0316	2.3957	0.8381	1.5212
	137	379.13	0.0114	12.8092	3.2905	1.0865	1.4595	0.5992	0.8541
	151	406.68	0.0175	13.9633	3.4626	1.1218	1.3925	0.6437	0.6806
	173	400.92	0.0180	17.1222	3.3510	1.3441	2.3214	0.7690	1.5008
	187	410.59	0.0165	15.7877	3.3114	1.1749	1.7595	0.8567	0.8370
	209	401.21	0.0197	14.3649	3.2537	1.0783	2.3341	0.7083	1.5676
	223	432.78	0.0257	16.3955	3.6119	1.1595	2.1246	0.8580	1.1841
	245	438.56	0.0183	15.5896	3.2932	1.1489	2.6069	0.8628	1.6894
	259	460.27	0.0199	17.4988	3.3349	1.0782	2.8428	1.1365	1.6298
5	9	382.99	0.0216	16.1674	1.6187	0.5705	2.0461	0.8226	1.2028
	27	388.20	0.0200	14.6469	3.2088	1.0667	1.7173	0.6126	1.0806
	45	396.19	0.0233	14.1790	3.3321	1.1178	1.7656	0.5367	1.1550
	63	382.60	0.0235	16.8021	2.5564	0.9985	1.9512	0.9019	0.9860
	81	413.33	0.0259	16.3192	4.1390	1.3833	2.3498	0.8773	1.3993
	99	424.45	0.0273	16.4627	3.6108	1.0819	1.9499	0.7651	1.1944
	117	421.39	0.0273	15.6476	3.1853	1.1310	1.6950	0.7487	0.9272
	135	423.19	0.0269	15.5878	3.1147	1.0603	1.7137	0.5536	1.1170
	153	414.53	0.0338	16.1636	3.0837	1.1002	2.3445	0.9285	1.3743
	171	427.32	0.0385	15.1192	3.5304	1.1508	2.5155	0.9280	1.5405
	189	415.24	0.0291	18.7063	3.3736	1.2089	2.4035	0.9517	1.4043
	207	429.87	0.0302	16.4704	3.8729	1.1282	2.3172	0.7617	1.4617
	225	447.74	0.0361	17.0420	3.4796	1.2149	2.0987	0.7493	1.3136
	243	415.23	0.0272	15.3013	2.9990	0.9776	2.2904	0.8632	1.3439
	261	394.28	0.0258	13.8748	3.5178	1.1241	1.9228	0.5987	1.2956
6	11	408.80	0.0151	13.2011	3.4650	1.0554	2.2420	0.9536	1.2537
	25	412.42	0.0169	14.6370	3.1591	1.2177	2.9383	0.8745	2.0067
	47	430.29	0.0152	14.4136	3.7433	1.2049	2.5687	1.0818	1.4565
	61	416.56	0.0160	14.3337	3.4632	1.2621	2.5725	0.9855	1.5229
	83	444.23	0.0166	18.1027	3.0594	1.1547	1.8081	1.0125	0.7405
	97	447.70	0.0242	16.9380	3.3140	1.1949	2.3581	0.8165	1.4884
	119	440.41	0.0201	16.0116	3.1083	1.1119	2.4020	1.0276	1.1765
	133	438.52	0.0139	14.6953	3.9345	1.4610	2.2810	0.9018	1.3201
	155	439.33	0.0181	16.1121	3.6630	1.3679	2.5495	0.9171	1.6009
	169	448.05	0.0251	15.1055	2.9616	1.2251	2.3606	1.1458	1.1677
	191	473.27	0.0158	18.1903	2.7355	0.9528	2.4310	0.7895	1.5747
	205	480.58	0.0165	19.5580	3.0873	1.1171	2.1534	0.9789	1.0601
	227	464.55	0.0215	18.7829	3.1785	1.0725	2.1273	0.8757	1.1753
	241	449.82	0.0169	16.0244	3.4524	1.1484	2.3473	0.7412	1.5841
	263	482.46	0.0206	19.3988	3.3246	1.2489	2.7400	1.0203	1.6795

(continued)

Table A-5. Individual Male Sacrifice and Organ Weights (g) (page 3 of 4)

Group[a]	Male ID	Sacrifice Weight	Thyroid	Liver	Paired Testis	Paired Epididymis	Accesory Sex Gland Unit	Prostate	Seminal Vesicles with Coagulating Glands
7	13	408.86	0.0169	16.6728	2.9816	1.0402	2.6443	0.8447	1.7073
	23	409.28	0.0242	15.9951	3.0491	1.1223	2.0609	1.0557	0.8425
	49	415.97	0.0136	15.9454	3.1117	1.0906	2.1119	0.7610	1.2995
	59	418.01	0.0176	15.8962	3.2465	1.1916	1.8878	1.0795	0.7389
	85	439.24	0.0159	16.4641	3.3450	1.1563	2.5087	0.8978	1.5688
	95	407.71	0.0124	14.1186	3.1467	1.1948	2.2663	1.0148	1.2263
	121	410.29	0.0166	16.5182	3.1562	1.2892	1.7974	0.8799	0.8718
	131	444.78	0.0151	15.0580	3.5558	1.1186	2.0418	0.6124	1.4084
	157	431.70	0.0212	17.6840	2.9147	1.1618	2.1445	1.0099	1.0227
	167	444.68	0.0173	17.9300	3.6797	1.2133	1.9658	0.6866	1.2488
	193	445.64	0.0162	18.4723	3.4596	1.1724	2.6683	1.1867	1.4398
	203	452.45	0.0183	17.2009	3.1164	1.0656	1.9838	0.6942	1.1789
	229	438.82	0.0176	16.7502	3.0104	1.0787	1.7386	0.5649	1.1472
	239	442.16	0.0213	17.5728	3.6511	1.3578	2.7774	1.3828	1.2455
	265	459.99	0.0172	15.7685	3.1771	1.1158	2.1017	0.5578	1.4848
8	15	377.72	0.0154	13.6036	2.8411	0.9739	1.3255	0.5390	0.7654
	21	390.56	0.0152	15.6927	3.2865	1.0876	1.8648	0.7332	1.1035
	51	376.30	0.0185	15.2001	2.8748	1.0065	1.4381	0.6784	0.7345
	57	411.32	0.0159	14.4837	3.1163	1.0037	1.7912	0.5669	1.1920
	87	414.97	0.0200	15.3183	3.3099	1.2109	2.1932	0.7975	1.3563
	93	421.42	0.0150	15.8493	3.4746	1.1175	2.4810	1.2052	1.1555
	123	419.76	0.0135	15.4618	3.1086	1.0429	1.9876	0.7466	1.1857
	129	390.89	0.0144	13.4963	3.7088	1.1060	1.7874	0.7479	1.0346
	159	404.58	0.0134	13.8364	3.4868	1.1485	2.2672	0.8628	1.3788
	165	418.09	0.0185	16.9881	3.5959	1.1239	1.9589	0.7188	1.1973
	195	424.84	0.0184	16.3084	3.5814	1.2513	2.2687	0.8827	1.3153
	201	445.55	0.0127	17.9894	3.2415	1.1259	2.0402	0.7031	1.2989
	231	430.25	0.0106	15.6910	3.3056	1.1062	2.1880	0.8613	1.2857
	237	435.45	0.0129	17.0311	3.0927	1.1243	2.4350	0.8172	1.5672
	267	461.39	0.0122	17.4283	2.9332	0.9813	1.9071	0.8840	0.8805
9	17	370.92	0.0292	13.1408	3.3825	1.1921	1.2820	0.6808	0.5782
	19	379.83	0.0271	13.9446	3.3301	1.0796	1.5685	0.5092	1.0284
	53	390.45	0.0268	13.3982	3.3357	1.0691	2.0327	0.7789	1.2070
	55	385.77	0.0348	13.6902	3.5586	1.1707	1.7429	0.6854	0.9832
	89	374.53	0.0316	14.4882	3.4041	1.0805	1.1964	0.5839	0.6010
	91	441.05	0.0283	18.1378	3.0158	1.1708	2.3377	0.9547	1.3522
	125	422.48	0.0242	15.5207	3.3391	1.2684	2.4969	0.8266	1.6228
	127	404.90	0.0257	15.2978	3.5066	1.2347	1.5672	0.7448	0.7951
	161	419.46	0.0319	15.7344	3.6707	1.1951	1.9308	0.6790	1.2050
	163	436.04	0.0229	17.1722	3.4924	1.2147	2.2050	1.0201	1.1074
	197	421.80	0.0216	16.7554	3.0358	1.1414	1.9821	0.5976	1.3410
	199	445.07	0.0255	19.2300	3.3251	1.1146	1.4748	0.6565	0.7934
	233	410.79	0.0328	13.2910	3.3559	1.2172	2.1179	1.0724	1.0086
	235	443.15	0.0245	16.1331	3.1717	1.0073	2.1659	0.7150	1.3907
	269	465.20	0.0363	18.8900	.[b]	1.1139	2.1981	0.9240	1.2318

(continued)

Table A-5. Individual Male Sacrifice and Organ Weights (g) (page 4 of 4)

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.
[b]Organ weight was a statistical outlier and therefore it was excluded.

Table A-6. Individual Male Hormone Data (page 1 of 5)

Group[a]	Male ID	Serum Testosterone[b]	Estradiol[c]	Follicle Stimulating Hormone[b]	Luteinizing Hormone[b]	Thyroid Stimulating Hormone[b]	Thyroxine[d]	Triiodo-thyronine[e]	Prolactin[b]	Dihydro-testosterone[c]
1	1	1.54	59.37	15.91	2.91	11.43	5.14	74.66	44.33	73.99
	35	1.49	40.59	15.77	2.87	10.79	4.43	82.70	48.95	67.65
	37	1.05	48.44	15.39	2.54	9.73	4.35	82.83	22.86	54.11
	71	0.22	43.84	15.65	5.00	9.57	3.17	68.73	102.70	45.39
	73	1.85	41.49	17.58	6.49	11.50	4.42	116.30	130.57	76.56
	107	1.50	40.69	15.68	2.57	10.74	3.65	83.68	71.29	67.41
	109	1.05	37.58	15.23	1.91	12.62	3.47	38.57	36.31	68.50
	143	2.05	40.25	16.07	2.44	24.24	3.49	94.30	62.83	100.52
	145	1.59	36.82	17.39	3.27	24.30	3.19	66.70	77.84	77.33
	179	0.74	39.34	16.60	3.52	8.65	4.59	73.22	42.57	57.80
	181	0.70	41.60	16.02	2.85	10.18	4.81	73.83	91.25	70.53
	215	2.87	38.74	14.69	3.19	8.72	6.08	114.94	46.29	189.79
	217	0.71	32.46	15.89	2.61	8.83	4.36	67.28	72.52	52.35
	251	1.98	34.46	17.71	3.66	15.04	4.06	59.83	37.20	92.66
	253	1.20	36.88	17.30	2.46	8.63	3.54	78.52	14.69	72.64
2	3	0.22	57.20	15.46	4.16	15.09	3.42	65.35	46.39	39.02
	33	6.12	50.51	18.61	7.44	13.32	3.71	51.32	56.49	247.34
	39	3.44	47.47	17.22	9.08	17.01	4.08	119.42	77.93	220.22
	69	0.36	54.07	10.96	2.91	7.44	3.95	86.19	48.46	43.68
	75	0.16	57.57	15.46	2.64	11.50	3.49	92.76	47.03	38.50
	105	4.25	47.24	16.52	3.74	11.07	3.70	73.54	57.06	163.46
	111	1.72	44.05	16.22	3.66	8.27	3.98	77.70	42.52	117.10
	141	2.83	47.37	16.12	4.29	8.68	2.98	93.80	56.18	91.17
	147	2.53	49.25	16.61	3.92	10.06	4.79	118.70	24.64	155.09
	177	0.28	45.91	15.61	3.58	16.37	2.16	70.17	56.76	45.26
	183	3.69	49.82	17.17	3.67	10.36	4.11	86.17	53.43	218.28
	213	1.34	56.18	16.61	4.47	9.07	4.23	118.12	17.34	74.68
	219	1.25	43.71	15.78	2.57	8.81	3.93	76.35	96.87	67.77
	249	0.63	47.53	16.31	2.86	11.66	2.61	64.52	8.14	44.59
	255	0.69	49.84	15.42	1.90	8.28	2.21	64.31	43.98	55.84

(continued)

Table A-6. Individual Male Hormone Data (page 2 of 5)

Group[a]	Male ID	Serum Testosterone[b]	Estradiol[c]	Follicle Stimulating Hormone[b]	Luteinizing Hormone[b]	Thyroid Stimulating Hormone[b]	Thyroxine[d]	Triiodo-thyronine[e]	Prolactin[b]	Dihydro-testosterone[c]
3	5	0.96	67.47	18.00	7.67	10.46	2.50	49.05	46.12	58.03
	31	1.98	62.88	19.69	8.10	8.41	2.85	69.74	24.78	122.00
	41	2.49	63.24	16.15	4.19	17.93	2.84	67.72	23.59	120.72
	67	0.18	47.18	18.20	1.87	9.09	3.07	70.79	62.56	62.90
	77	0.87	52.94	17.38	2.81	8.50	2.62	71.22	7.91	52.51
	103	5.40	60.95	16.89	3.46	9.14	3.47	80.13	48.06	273.92
	113	0.58	63.30	17.74	3.81	7.81	2.08	62.60	41.46	57.04
	139	1.54	61.50	17.38	3.42	11.03	3.11	104.43	41.54	79.55
	149	1.39	56.73	16.34	5.44	9.76	3.62	91.67	38.02	111.31
	175	3.23	61.15	16.11	3.74	8.10	2.55	49.11	38.85	126.00
	185	0.32	86.19	16.92	2.48	9.50	1.73	86.51	76.69	55.27
	211	1.42	47.26	17.52	2.70	19.67	2.38	57.15	33.84	115.50
	221	1.48	54.92	17.44	4.64	9.26	2.08	56.56	59.05	94.64
	247	0.59	61.96	14.97	2.84	10.72	2.54	83.60	56.33	49.91
	257	1.27	70.15	16.83	2.28	9.42	1.56	63.30	39.67	75.80
4	7	0.18	74.46	16.66	5.14	10.33	2.25	64.66	40.15	45.02
	29	0.72	73.63	15.18	3.05	10.24	2.81	66.53	90.18	63.97
	43	0.88	74.72	17.04	7.26	9.62	3.81	94.42	56.05	68.93
	65	0.10	73.71	17.23	3.96	9.34	2.33	49.14	72.13	34.98
	79	1.48	53.63	14.16	7.74	9.08	2.34	73.58	58.54	85.36
	101	1.11	59.81	14.22	5.21	7.71	2.38	82.17	42.06	67.37
	115	0.22	75.64	15.47	2.34	6.34	2.35	70.72	41.77	43.22
	137	1.16	76.54	15.97	1.60	12.20	2.43	95.19	19.24	69.67
	151	0.17	52.68	14.76	3.43	7.16	2.71	89.64	48.71	58.13
	173	0.27	49.27	14.88	2.87	7.55	1.89	63.57	46.39	51.15
	187	0.17	56.42	16.32	3.51	11.62	2.31	73.07	49.17	38.47
	209	1.76	55.57	17.26	3.36	9.33	2.60	88.57	30.74	84.99
	223	0.96	70.85	19.09	5.49	11.16	2.55	90.32	61.87	57.50
	245	1.40	60.18	19.69	2.41	9.98	2.22	52.44	20.35	79.15
	259	2.41	48.97	18.80	3.05	7.36	2.79	76.49	30.17	119.47

(continued)

Table A-6. Individual Male Hormone Data (page 3 of 5)

Group[a]	Male ID	Serum Testosterone[b]	Estradiol[c]	Follicle Stimulating Hormone[b]	Luteinizing Hormone[b]	Thyroid Stimulating Hormone[b]	Thyroxine[d]	Triiodo-thyronine[e]	Prolactin[b]	Dihydro-testosterone[c]
5	9	0.58	74.65	17.21	2.97	13.46	2.44	87.58	31.21	53.23
	27	2.40	65.98	15.28	2.44	9.17	1.69	62.38	8.02	112.68
	45	6.98	62.76	18.97	5.80	9.35	3.17	74.82	39.08	275.79
	63	1.47	69.21	17.26	3.28	11.29	2.01	77.85	65.94	137.73
	81	0.91	86.98	16.08	2.59	10.64	2.02	105.60	49.50	90.07
	99	1.40	83.42	14.63	4.63	9.10	1.68	98.61	37.33	54.43
	117	0.50	59.01	13.58	1.56	10.27	1.60	57.73	29.93	49.32
	135	1.23	64.81	13.78	4.02	7.02	1.72	99.05	35.01	81.90
	153	2.42	58.38	16.41	2.66	10.22	2.43	85.66	9.47	172.77
	171	1.33	78.37	16.11	2.64	8.65	1.56	60.84	35.31	84.09
	189	0.79	54.97	15.53	2.75	8.57	2.36	108.11	23.86	61.89
	207	2.62	49.23	17.00	5.35	13.36	2.64	72.13	28.96	196.27
	225	2.88	63.85	16.96	2.74	19.25	1.86	66.31	44.07	103.28
	243	0.69	48.78	15.76	2.19	9.14	1.37	71.24	5.93	64.88
	261	0.31	63.24	17.12	2.22	9.62	1.56	62.24	49.27	43.35
6	11	8.04	55.47	17.37	4.42	8.70	5.48	66.15	22.83	286.77
	25	3.66	50.02	16.65	3.47	7.50	4.31	62.98	54.07	140.38
	47	1.76	58.30	15.29	5.15	12.30	3.65	83.10	48.36	93.81
	61	0.15	45.93	16.97	3.78	10.24	4.74	84.50	94.02	40.73
	83	3.41	44.78	16.42	2.47	11.60	5.05	115.43	91.91	172.09
	97	0.80	38.21	15.22	2.40	9.95	4.58	97.35	99.50	65.50
	119	1.63	46.01	17.30	3.70	8.38	4.69	72.64	44.16	126.32
	133	0.82	37.54	14.86	3.37	7.61	3.61	91.91	40.49	63.29
	155	3.06	36.83	20.63	3.65	9.64	4.93	97.77	25.06	140.92
	169	1.23	41.32	15.84	2.58	8.04	4.18	61.47	123.21	60.74
	191	0.44	35.07	17.15	4.40	13.58	3.63	49.47	42.43	48.96
	205	0.51	29.49	14.96	3.58	13.60	5.28	86.09	53.92	52.45
	227	0.43	33.50	14.81	2.73	11.63	3.81	53.77	41.16	46.55
	241	1.65	40.59	16.14	2.42	7.83	3.75	63.82	10.11	72.87
	263	0.29	40.10	17.21	2.20	21.52	5.17	89.73	9.92	50.49

(continued)

Table A-6. Individual Male Hormone Data (page 4 of 5)

Group[a]	Male ID	Serum Testosterone[b]	Estradiol[c]	Follicle Stimulating Hormone[b]	Luteinizing Hormone[b]	Thyroid Stimulating Hormone[b]	Thyroxine[d]	Triiodo-thyronine[e]	Prolactin[b]	Dihydro-testosterone[c]
7	13	1.37	40.49	17.14	4.69	15.12	5.15	94.73	154.90	54.47
	23	3.44	45.69	15.50	3.16	14.45	4.78	99.18	82.67	196.21
	49	1.63	43.68	17.21	3.17	17.02	5.26	94.18	110.75	90.89
	59	0.19	57.92	15.91	3.41	14.71	6.05	96.42	174.36	39.12
	85	6.14	54.84	18.04	3.77	11.56	5.91	96.57	191.61	284.71
	95	2.52	49.69	15.71	2.69	17.10	3.15	91.04	94.04	144.49
	121	0.36	45.57	16.03	3.11	18.62	5.45	87.71	277.20	36.21
	131	0.79	55.70	16.11	2.97	7.40	3.60	54.16	47.14	50.75
	157	1.23	41.88	16.48	3.90	8.58	5.11	96.06	128.37	109.61
	167	1.12	42.38	15.09	2.73	11.85	5.22	76.40	113.83	67.58
	193	1.68	42.11	15.65	2.76	11.94	5.43	74.26	51.50	85.40
	203	1.35	43.71	17.72	3.86	13.52	5.79	87.21	111.38	88.33
	229	0.57	38.61	17.82	2.73	10.80	5.62	76.14	120.95	52.41
	239	2.46	33.84	14.20	2.17	9.44	4.14	72.76	25.26	105.28
	265	1.40	37.91	15.20	2.33	9.78	4.71	65.38	96.74	98.50
8	15	0.53	67.17	15.19	1.78	9.44	3.88	68.84	39.36	56.72
	21	1.15	61.99	16.48	4.67	14.56	5.06	87.00	110.72	48.07
	51	0.08	38.45	15.22	4.06	10.55	4.71	83.73	90.47	39.81
	57	0.33	47.93	14.14	3.12	11.59	5.21	62.14	153.12	50.49
	87	1.17	45.75	15.39	5.88	15.93	4.14	96.20	111.56	77.23
	93	0.84	41.40	18.02	5.35	17.54	4.89	119.13	45.55	39.73
	123	0.80	43.16	14.82	3.52	14.36	4.74	85.11	46.36	63.14
	129	0.07	46.96	16.28	3.56	9.37	3.44	48.83	72.30	33.75
	159	2.11	54.34	18.32	4.74	6.86	5.86	102.53	64.01	148.81
	165	3.50	46.96	15.42	2.94	14.50	4.94	58.09	315.97	180.65
	195	1.36	47.08	16.48	4.82	9.32	3.86	69.64	85.17	92.75
	201	0.54	36.01	18.47	2.47	9.71	4.84	76.00	37.28	65.43
	231	0.16	48.27	15.35	3.22	10.98	4.10	78.52	167.40	31.09
	237	1.69	37.23	17.54	3.38	9.00	3.83	82.02	34.43	72.65
	267	1.79	34.16	14.99	3.16	9.33	4.22	75.77	53.15	68.23

(continued)

Table A-6. Individual Male Hormone Data (page 5 of 5)

Group[a]	Male ID	Serum Testosterone[b]	Estradiol[c]	Follicle Stimulating Hormone[b]	Luteinizing Hormone[b]	Thyroid Stimulating Hormone[b]	Thyroxine[d]	Triiodo-thyronine[e]	Prolactin[b]	Dihydro-testosterone[c]
9	17	0.23	66.17	14.20	3.40	11.47	4.83	75.93	236.98	53.55
	19	2.37	49.91	16.10	4.63	10.63	4.97	69.09	60.35	103.33
	53	0.34	54.20	15.06	2.28	29.58	5.74	76.05	166.56	40.53
	55	2.40	64.84	17.34	6.06	11.95	5.91	76.01	214.52	117.25
	89	0.28	37.34	17.64	5.25	11.38	4.43	64.35	215.54	43.00
	91	1.31	57.04	15.87	2.04	21.13	5.12	107.89	49.66	100.53
	125	1.18	33.74	19.05	3.00	12.07	5.21	81.89	115.76	71.30
	127	0.28	57.87	16.49	4.29	12.95	3.75	79.70	229.45	35.91
	161	1.62	41.75	16.02	2.67	9.29	6.09	85.81	117.53	99.29
	163	0.32	41.90	15.86	3.23	9.81	4.36	56.46	222.16	46.81
	197	0.35	43.88	13.83	2.43	14.12	4.19	80.48	174.41	55.97
	199	2.68	34.91	14.70	3.68	8.71	4.17	74.52	119.37	136.95
	233	0.54	42.56	17.10	5.88	8.79	3.30	54.92	145.63	39.62
	235	0.13	45.24	16.44	2.62	7.89	4.51	68.42	165.55	34.78
	269	1.31	40.24	16.42	3.04	10.03	5.40	84.81	83.55	67.87

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.
[b]Unit of measure is ng/ml.
[c]Unit of measure is pg/ml.
[d]Unit of measure is ug/dL.
[e]Unit of measure is ng/dL.

Table A-7. Individual Male Gross Necropsy Findings (page 1 of 1)

Group[a]	Male ID	Finding
3	31	Testis: reduced in size, bilateral Epididymis: reduced in size, bilateral
4	151	Seminal Vesicles: reduced in size Prostate: reduced in size
5	9	Testis: reduced in size, bilateral
	63	Testis: reduced in size, bilateral Epididymis: reduced in size, bilateral
9	89	Seminal Vesicles: reduced in size Prostate: reduced in size
	199	Seminal Vesicles: reduced in size Prostate: reduced in size

[a]Groups are as follows: 1 is control; 2 is 25 mg/kg/day of Linuron; 3 is 50 mg/kg/day of Linuron; 4 is 75 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; 6 is 12.5 mg/kg/day of Methoxychlor; 7 is 25 mg/kg/day of Methoxychlor; 8 is 37.5 mg/kg/day of Methoxychlor; and 9 is 50 mg/kg/day of Methoxychlor.

RTI INTERNATIONAL

Quality Assurance Statement

Study Title: 15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley CD® Rats

Sponsor: Battelle Memorial Institute

Study Code: Rt03-ED07

Protocol Number: RTI-871

This study was audited by the Sciences and Engineering – Health Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader and Management
Protocol Audit	December 3-4, 2002	December 11, 2002
Dosing	May 19, 2003	May 19, 2003
Hormone Analysis	September 16. 2003	September 16, 2003
Necropsy	June 5, 2003	June 16, 2003
Data Audit	September 11-12, 15-17, 19, 22-23, 2003	October 6, 2003
Data Audit	February 12, 2004	February 16, 2004
Data Audit	November 11-14, 17-19, 21, 24-26 December 1-4, 8-12, 15, 18, 24, 29, 30, 2003 January 7-9, 2004	January 9, 2004
Data Audit	January 14-15, 2004	January 15, 2004
Report Audit	March 2-3, 2004	March 3, 2004
Report Audit	February 12 – March 10, 2004	March 11, 2004

Prepared by: Michelle Oh — 04/25/05

Michelle Oh
Quality Assurance Specialist

Date

Reviewed by: Carrie Ingalls — 04/25/05

Carrie Ingalls
Quality Assurance Assistant Manager

Date

FINAL REPORT
APPENDICES

Title: 15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley (CD®) Rats

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Battelle Memorial Institute

Study Initiation Date: February 12, 2003

In-Life Performance Dates: May 19, 2003– June 6, 2003

Audited Final Report Date: April 25, 2005

RTI Identification Number: 65U-08055.001.021

APPENDIX 1

Histopathology Report

15-DAY TIER 1 SCREEN OF ENDOCRINE ACTIVE COMPOUNDS ADMINISTERED BY GAVAGE TO ADULT MALE SPRAGUE-DAWLEY (CD®) RATS

CLIENT ID: 65U-08055.001.021
EPL PROJECT NO. 237-007

PATHOLOGY REPORT

Submitted to

Research Triangle Institute
P.O. Box 12194
Research Triangle Park, NC 27709

Submitted by

Experimental Pathology Laboratories, Inc.
P. O. Box 12766
Research Triangle Park, NC 27709

March 24, 2005

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15-DAY TIER 1 SCREEN OF ENDOCRINE ACTIVE COMPOUNDS ADMINISTERED BY GAVAGE TO ADULT MALE SPRAGUE-DAWLEY (CD®) RATS

65U-08055.001.021

EPL PROJECT NO. 237-007

NARRATIVE SUMMARY

INTRODUCTION

The objectives of this study were to:

1. Evaluate the design of an *in vivo* screen for identifying the mode of action of potential endocrine modulators in adult male rats, using a collection of endpoints;
2. Characterize the response of this endocrine screen using two compounds with known endocrine activity.

   For this study, Linuron and Methoxychlor were tested. The testes, epididymides and thyroids were examined microscopically.

SUMMARY

Administration of the test chemicals by gavage to male Sprague-Dawley (CD®) rats, under the conditions of this study, was associated with the following histopathologic changes:

1. Minimal seminiferous tubule degeneration of the testis and exfoliated germ cells in the epididymides were noted in some high-dose Methoxychlor animals.
2. No unequivocal exposure-related changes were observed in the high-dose Linuron males.

DESIGN OF THE STUDY

Linuron and Methoxychlor were administered via gavage once daily for 15 consecutive days to male Sprague-Dawley (CD®) rats under the conditions outlined in the study protocol (RTI Master Protocol No.: RTI-871).

The study began with 15 weight-matched males/group. The study design, test chemicals and target dose levels are presented in Table 1.

Table 1 – Study Design

Group No.	Number of Males	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
COMPONENT 1					
1	15	-[a]	0	0.0	5
2	15	Linuron	25	5.0	5
3	15		50	10.0	5
4	15		75	15.0	5
5	15		100	20.0	5
6	15	Methoxychlor	12.5	2.5	5
7	15		25	5.0	5
8	15		37.5	7.5	5
9	15		50	10.0	5

[a] 0.25% aqueous methylcellulose, vehicle control

Individual treatment groups within the study were given unique five digit codes that are presented in Table 2

Table 2 – Treatment Group Designations

Group (mg/kg)	Dose Codes
0	09560
Linuron – 25	59698
Linuron – 50	69300
Linuron – 75	80588
Linuron – 100	71418
Methoxychlor – 12.5	35613
Methoxychlor – 25	43804
Methoxychlor – 37.5	20565
Methoxychlor - 50	18811

According to the study protocol, tissues taken at necropsy were placed in fixative and transferred to Experimental Pathology Laboratories, Inc. (EPL®) for processing. The thyroids (with trachea attached), liver and one epididymis were placed in 10% neutral buffered formalin. The testes were placed in Bouin's fixative for 24 hours and then stored in 70% alcohol. All tissues were trimmed, embedded in paraffin, sectioned and stained with Hematoxylin & Eosin (H&E).

The thyroids were weighed post-fixation at EPL.

Histopathological examination of selected organs was conducted on the protocol-required tissues. The protocol-required tissues were: testes, epididymides and thyroid glands.

The gross and histopathologic data were entered in EPL's Computerized Pathology Reporting System. Each lesion was graded according to a four-grade severity code (1-4).

RESULTS

The individual animal data are presented by group in the Histopathology Incidence Tables (HIT) and the group summary data in the Summary Incidence Tables (SIT). Gross necropsy findings were correlated to the microscopic findings, whenever possible. These findings are presented in the section "Correlation of Gross and Microscopic Findings Tables".

According to the body and organ weight data, the body weights of both the Linuron and Methoxychlor-exposed animals decreased. Although some absolute and relative organ weights decreased or on occasion increased, most weighed tissues were not examined microscopically. No related histopathology was detected in the testes, epididymides or thyroid glands which could account for any of the weight changes observed.

TREATMENT-RELATED FINDINGS BY CHEMICAL

Methoxychlor

Administration of 50 mg/kg Methoxychlor was associated with the increased incidence of testicular seminiferous tubule degeneration in a little over one-third of the animals examined.

Seminiferous tubule degeneration was characterized by a spectrum of very subtle changes which included vacuolization within the germinal epithelium lining the tubule, single cell degeneration to necrosis of germinal epithelial cells, desquamated germ cells into the tubule lumen and, on occasion, sperm retention in stage IX. At this dose, all of the degenerative lesions were graded as minimal which indicated they were present in only one to two tubules up to fewer than 10% of the tubules present in any testis cross-section. In support of seminiferous

tubule degeneration, increased numbers of exfoliated germ cells were present in epididymal tubules. Normally, low numbers of exfoliated germ cells may be observed in epididymal tubules, but these numbers can increase with seminiferous tubule degeneration. The pathogenesis and significance of the seminiferous tubule degeneration remains unclear at this time. In a related study with Methoxychlor administered at the same dose level, no exposure-related testicular lesions were noted (Pathology Report 65U-08055.001.015.001(M), EPL Project No. 237-006).

The incidence and severity of seminiferous tubule degeneration in the Methoxychlor males is presented in Table 3.

Table 3 – Incidence and Severity of Seminiferous Tubule Degeneration

Dose (mg/kg)	0	50
TESTIS (No. Examined)	(15)	(15)
Degeneration, Seminiferous Tubule	1	6
Minimal	1	6

Linuron

Treatment-associated lesions were not observed in the Linuron-exposed animals. One animal was noted to have seminiferous tubule degeneration which was graded as moderate. However in the experience of the reviewing pathologist, similar lesions may be, on occasion, observed in control animals as well.

John Curtis Seely, DVM

JOHN CURTIS SEELY, D.V.M.
Diplomate, ACVP
Senior Pathologist

March 24, 2005
Date

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: 15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley (CD®) Rats

Client Study: 65U-08055.001.021; Rt03-ED07; RTI-871

EPL Project Coordinator: Dr. John Curtis Seely

EPL Project Number: 237-007

EPL Pathologist: Dr. John Curtis Seely

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

Area Inspected	Dates Inspection	Dates Reporting
EPL Project Sheets	June 25, 2003; July 8, 2003	June 25, 2003; July 8, 2003
Project Setup	July 1, 2003	July 1, 2003
Histology Setup	July 1, 2003	July 1, 2003
Data Review	July 10, 2003; July 14, 2003	July 10, 2003; July 14, 2003
Draft Report	December 2&3, 2003	December 3, 2003
Final Report	March 24, 2005	March 24, 2005

Date reported to Study Director/Management: December 29, 2003; March 24, 2005

Date of last quarterly facility inspection: January 2005

Jane J. Hollingsworth
EPL Quality Assurance Unit

March 24, 2005
Date

15-DAY TIER 1 SCREEN OF ENDOCRINE ACTIVE COMPOUNDS ADMINISTERED BY GAVAGE TO ADULT MALE SPRAGUE-DAWLEY (CD®) RATS

65U-08055.001.021

EPL PROJECT NO. 237-007

Study Design

Group No.	Number of Males	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
COMPONENT 1					
1	15	-[a]	0	0.0	5
2	15	Linuron	25	5.0	5
3	15		50	10.0	5
4	15		75	15.0	5
5	15		100	20.0	5
6	15	Methoxychlor	12.5	2.5	5
7	15		25	5.0	5
8	15		37.5	7.5	5
9	15		50	10.0	5

[a] 0.25% aqueous methylcellulose, vehicle control

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

Rt03-ED07
15-Day Sacrifice
Male Rat

	GROUP 09560	GROUP 71418	GROUP 18811			
EPIDIDYMIS (NO. EXAMINED)	(15)	(15)	(15)			
Exfoliated Germ Cells, Lumen		1	6			
TESTIS (NO. EXAMINED)	(15)	(15)	(15)			
Degeneration, Seminiferous Tubule	1	1	6			
THYROID (NO. EXAMINED)	(15)	(15)	(15)			

HISTOPATHOLOGY INCIDENCE TABLES

HISTOPATHOLOGY INCIDENCE TABLE

Rt03-ED07
15-Day Sacrifice
Male Rat

GROUP
09560

ANIMAL	1	35	37	71	73	107	109	143	145	179	181	215	217	251	253
EPIDIDYMIS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exfoliated Germ Cells, Lumen															
TESTIS	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Degeneration, Seminiferous Tubule												1			
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

HISTOPATHOLOGY INCIDENCE TABLE

Rt03-ED07
15-Day Sacrifice
Male Rat

GROUP
71418

ANIMAL	9	27	45	63	81	99	117	135	153	171	189	207	225	243	261
EPIDIDYMIS		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exfoliated Germ Cells, Lumen	2														
TESTIS		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Degeneration, Seminiferous Tubule	3														
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

Rt03-ED07
15-Day Sacrifice
Male Rat

GROUP
18811

ANIMAL	17	19	53	55	89	91	125	127	161	163	197	199	233	235	269
EPIDIDYMIS	X	X		X						X	X	X	X	X	X
Exfoliated Germ Cells, Lumen			2		1	1	1	1	1						
TESTIS	X	X		X			X			X	X	X	X		X
Degeneration, Seminiferous Tubule			1		1	1		1	1					1	
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

Rt03-ED07
15-Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 71418

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
9	TESTIS	Reduced in size, bilateral	Degeneration, Seminiferous Tubule
63	TESTIS	Reduced in size, bilateral	No Correlating Lesion
	EPIDIDYMIS	Reduced in size, bilateral	No Correlating Lesion

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 18811

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
89	SEMINAL VESICLE	Reduced in size	Intentionally Not Sampled
	PROSTATE	Reduced in size	Intentionally Not Sampled
199	SEMINAL VESICLE	Reduced in size	Intentionally Not Sampled
	PROSTATE	Reduced in size	Intentionally Not Sampled

Appendix II

Analytical Chemistry Reports

Battelle
Putting Technology To Work

Chemical Repository Services for the EDSP
EPA Contract No. 68-W-01-023

Chemistry Report for WA 2-28
Linuron in a Methylcellulose and Water Suspension

Revised: March 24, 2005

Prepared By:		Approved By:	
Eric A. Crecelius	3/24/05	RM Ecker	3/24/05
Eric A. Crecelius, Ph.D. Chemical Repository Manager	Date	Richard M. Ecker Director, Marine Sciences Laboratory	Date

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
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Submitted to:

Dr. Julia George

Center for Life Sciences and Toxicology
Research Triangle Institute

PO Box 12194

Research Triangle Park, NC 27709

Chemistry Report for WA 2-28
Linuron in a Methylcellulose and Water Suspension

Reviewed by: Mary E Lynn

Mary E. Lynn, EDSP Quality Assurance Representative

Battelle Marine Sciences Laboratory

Date: 3/24/05

Chemistry Report for WA 2-28
Linuron in a Methylcellulose and Water Suspension

Parameter	Chemical	
Compound Name	Linuron	Linuron
CAS #	330-55-2	330-55-2
Central File No.	CF-1824	CF-2006
Initial Receipt Date	8/29/02	4/17/03
Expiration Date	11/05	4/07
Manufacturer	Chem Service	Chem Service
Lot Number	273-81B	301-91A
Battelle Study #	WA 2-28-03-01	WA 2-28-03-01
Method	SW 846, 8316 Modified	SW 846, 8316 Modified

Executive Summary

The chemical purity of linuron determined by the manufacturer was 99% for both lots (CAS 3350-55-2; lot numbers 273-81B expiration date 11/05 and 301-91A expiration date 4/07). The purity results of linuron from Battelle-Sequim by HPLC was determined to be 99.6% for CF-1824 (Lot No. 273-81B) and 99.9% for CF-2006 (Lot No. 301-91A). Stability testing was conducted on CF-1824 and in-life test solutions were made with CF-2006. Based on the final regression model and the lower 95% confidence limit of the slope, the concentration of linuron was expected to stay greater than or equal to 90% of the target concentration for up to an estimated 6.5 weeks. Thus, stability testing of the linuron stock solution in methylcellulose was considered stable at 5 mg/mL for the required holding period of 21 days.

Methylcellulose (CAS 9004-67-5, lot number 062K0144) was purchased from Sigma Aldrich 1/03 to be used as a carrier for the stability and biological testing. It was assigned a central file number of CF-1969 and an expiration date of 1/07. The carrier had no visual defects and was stored at room temperature.

In-life linuron concentrations had a mean of 7.19 and 15.4 mg/mL for the 10 and 15 mg/mL targets, respectively. Recoveries relative to the dosing target ranged from 31% to 130% for the 10 mg/mL target concentration and 40% to 174% for the 15 mg/mL target concentration during the course of the assay.

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1.0 INTRODUCTION

The goal of the Battelle-Sequim, Marine Sciences Laboratory (MSL) Chemical Repository for the Endocrine Disruptor Screening Program (EDSP) is to provide the participating laboratory with requested chemicals of documented quality at required concentrations and in a matrix appropriate for different toxicological tests. The EDSP Chemical Repository supplies the manufacturer's information regarding purity and stability, the material safety data sheet (MSDS) chemical information, and independent analysis of purity and stability in a matrix specified by the Purity and Stability Testing Plan made in collaboration with the requesting Principal Investigator. Additional analyses associated with the in-life studies are also provided when requested. This report is the product of such a request.

Under Work Assignment (WA) 2-28 and Battelle-Sequim Study Number WA 2-28-03-01, Dr. Julia George from Center for Life Sciences and Toxicology, Research Triangle Institute, requested purity and stability testing of linuron (Figure 1). Electronic files submitted to the EDSP Data Coordination Center in support of this work assignment were CRF_WA-2-28_linuron-mc.doc, PSTP_WA-2-28_linuron-methylcellulose.doc, DSUM_WA-2-28_Linuron-mc.xls, and DAF_WA-2-10_Linuron-mc.doc.

2.0 GENERAL METHODS

Methods of standard operation of the Chemical Repository are addressed in the procedure, EDSP.C-001-01, The EDSP Chemical Repository. This procedure addresses chemical procurement including procurement of controlled substances, when applicable, which have unique permitting, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage; stock solution preparation, documentation and archiving; test solution preparation, documentation and shipping; chemical disposal, and repository maintenance over time. The quality assurance (QA) requirements for procurement of chemicals for use in the Chemical Repository are addressed in procedure, MSL-A-012, Procurement. Each purchase requisition receives QA review to determine what is being ordered and which specific requirements apply.

2.1 Chemical Procurement

As requested by Dr. Julia George, linuron, (CAS No. 330-55-2) was purchased for purity and stability analysis for use in a bioassay on rats (Figure 1). Linuron was purchased from Chem Service and lot number 273-81B was received on 8/29/02 with an expiration date of 11/05 (Table 1). The chemical was left in the original container, logged in to the Chemical Management System (CMS) and given a CMS barcode and unique log in number (CF-1824) as per the QA Project Plan (QAPP) for the EDSP Chemical Repository. Additional linuron was purchased from Chem Service and lot number 301-91A was received on 4/17/03 with an expiration date of 4/07. The chemical was left in the original container and logged in as CF-2006. CF-1824 was used for stability testing and CF-2006 was used for biological testing. The chemical was stored in a dry location at room temperature, away from direct sunlight.

Methylcellulose (CAS 9004-67-5, lot number 062K0144) was purchased on 1/31/03 from Sigma, Inc., to be used as a carrier for both the stability and biological testing. The carrier had no visual defects and was stored at room temperature.

EDSP Chemical Request Form

For EPA WA: 2-28-03-01

Study Director

Name: Dr. Julia George
Affiliation: Center for Life Sciences and Toxicology
Research Triangle Institute
Location: PO Box 12194
Research Triangle Park, NC 27709
Telephone number: 919-541-5862

Bioassay Information

Proposed Bioassay: WA 2-28

Test Chemical: Linuron (MSL CF-1824)

Carrier(s): Methylcellulose

Concentrations/Dilution Series: 10 and 15 mg/mL

*Consider if analysis method detection limit which may be determined in
Purity analysis is above or below desired test concentrations?

In vitro or *in vivo* tests? *In vivo*

Organism to be tested: rat

Method of test solution administration: oral gavage

Testing/holding duration: 3 weeks

Chemical Information

Chemical Name: Linuron

CAS: 330-55-2

Any known purity information: may refer to attached documentation	Manufacturer's Purity Information: 99% pure
Any known stability information: may refer to attached documentation	None available
Desired purity (%) for test? 95% or greater	

Figure 1. EDSP Requisition Form for Linuron

Table 1. Chemical Procurement Information

Parameter	Chemical	
Compound Name	Linuron	Linuron
CAS #	330-55-2	330-55-2
Central File No.	CF-1824	CF-2006
Initial Receipt Date	8/29/02	4/17/03
Expiration Date	11/05	4/07
Manufacturer	Chem Service	Chem Service
Lot Number	273-81B	301-91A
Manufacturer's Purity	99%	99%
Storage Conditions	Dry place/room temp.	Dry place/room temp.
Battelle Study #	WA 2-28-03-01	WA 2-28-03-01
Method	SW 846, 8316 Modified	SW 846, 8316 Modified

2.2 Chemical Purity

Chemical purity was verified by chromatographic analysis to determine areas under peaks other than the principal peak and compared to the manufacturer's certificate of analysis/purity (Appendix A). No statistical analyses were performed for the verification of chemical purity. General methods are documented in the procedure, EDSP.D-012-01, Chemical Repository Summary Displays and Statistical Analyses for the EDSP Data Coordination Center (DCC).

The purity analysis was conducted on two lots of linuron; CF-1824, CAS 330-55-2, Chem Service lot number 273-81B, expiration date 11/05 and CF-2006, CAS 330-55-2, Chem Service lot number 301-91A, expiration date 4/07. Stability testing was conducted with CF-1824 and in-life test solutions were made with CF-2006.

Purity verification was conducted using a high performance liquid chromatograph (HPLC) with ultraviolet (UV) absorbance at 250 nm by taking a solution of about 5000 ng/mL in 60% acetonitrile: 40% de-ionized water solution. This matrix was then run on the HPLC and the purity determined by comparing the peak heights of the peaks in the chromatogram. The purity was determined by first identifying the peaks in the chromatogram of the linuron that were the same as the peaks in the analysis of the blank sample. The areas associated with these common peaks were then eliminated by inhibiting integration and the remaining peaks were reported as a percentage of the total peak area. The percentage associated with the largest peak represented the purity of linuron. The HPLC was set up with an autosampler. The autosampler was set to inject 150 μL of the matrix. One replicate was analyzed.

2.3 Preparation of Stock Matrices for Stability Analysis

A general study plan for stability testing based on the WA 2-28 request from Dr. Julia George was developed as the stability test protocol and is presented in Appendix B. A single stock solution was prepared to arrive at the chemical concentrations requested for stability analysis (Table 2). All samples were analyzed in triplicate so that a mean concentration and relative standard deviation (RSD) could be determined. General methods are documented in EDSP.D-012-01.

Linuron stock matrices were prepared for testing as described in Table 2. Briefly, the 0.25% methylcellulose solution suspension was prepared by adding 2.5 g of methylcellulose (CF

1969, CAS 9004-67-5, Sigma Aldrich lot # 062K0144, assigned expiration date 1/07) to 700 mL boiling reagent water while stirring. The heat was turned off and the solution stirred overnight then diluted to 1000 mL. The clear solution was stored at 4°C until use. On 3/19/03 the linuron plus methylcellulose suspension (5.0 mg/mL) was prepared by weighing out 2.0 g linuron (CF 1824-1, CAS 330-55-2, Chem Services lot number 273-81B, expiration date 11/1/05, that was previously sieved through 80 mesh) into an amber glass wide-mouth jar with 398 g of the methylcellulose solution. The mixture with a total volume of 400 mL was made in a 500 mL amber glass container, which was stirred overnight using a stir bar and a magnetic stirrer. The container was labeled and stored at 4ºC ± 2ºC for the duration of the test.

Two high concentration solutions were made to assess homogeneity. The first solution was made (4/25/03) with linuron that had not been sieved through a 500 μm screen before use, and there were problems analyzing the samples. A second 20 mg/mL solution was made on 5/6/03 using 8 g linuron (CF 1824-1, CAS 330-55-2, Chem Service lot #273-81B, expiration date 11/05) and 392 g of 0.25% methylcellulose solution from a preparation made on 5/5/03.

2.4 Analytical Chemistry for Stability Testing

Chemical stability was evaluated under storage conditions and matrix specifications as requested by the participating laboratory. At initiation and at each time period throughout the duration of the test, the chemical concentration was determined by chromatographic analysis. Triplicate aliquots were tested. The frequency of determinations and the duration of testing were determined by the requesting principal investigator and the chemists based on *a priori* knowledge about chemical stability. General methods are documented in EDSP.D-012-01.

Linuron stock solution was sampled for stability by removing a 1 mL sample from the top, middle, and bottom of the slurry using a 5 mL plastic needle and syringe. The 1 mL sample was placed into a tared 25 mL amber glass bottle, and the weight determined gravimetrically. The sample weight was determined by subtracting the tare weight of the bottle from the bottle plus sample weight. Then 25 mL acetonitrile (ACN, JT Baker lot # X44836) was added to the bottle, and the bottle was agitated for 1 minute. Next, 0.01 mL of the bottle solution was pipetted into an autosampler vial with 0.99 mL 60% ACN in water. The solution was analyzed by HPLC using an ultraviolet/visible (UV/VIS) wavelength detector at the 250 nm wavelength. Separation was attained using a Phenomenex Hydro C-18 column. Calibration was performed on 3/27/03 using dilutions prepared from the standard PP-1192 at 50, 200, 500, 2000, and 5000 ng/mL. HPLC runs are stored on the computer with the property number WV04738. The test was conducted for 19 days. A methylcellulose blank was prepared and analyzed in the same way. Continuing calibration verification (CCV) samples were analyzed to demonstrate ongoing calibration accuracy.

Table 2. Stock Matrix Composition for Stability Testing

Study and Duration	Test Chemical	Target Concentration	Sample ID	Stock Matrix
WA 2-28-03-01 3 Weeks	Linuron	5 mg/mL	1824-2a-3	2.0 g linuron in 398 mL methylcellulose water diluted to a final volume of 400 mL with deionized water

2.5 Statistical Analysis of Stability

Log linear degradation curves were fit to the data to describe the chemical concentration over time and their dependence on storage conditions and solvent matrix. Lack of fit and residual plots were evaluated to determine the form of the regression. Power calculations based on the observed variability were used to determine the sensitivity of the test to detect degraded concentrations. General methods are documented in SOP EDSP.D-012-01.

2.6 Analytical Chemistry for In-Life Testing

Analytical methods associated with in-life testing were similar to those described in Section 2.4.

3.0 RESULTS

3.1 Chemical Purity

Battelle-Sequim conducted a HPLC purity scan on the linuron. The purity analysis was conducted on two lots of linuron; CF 1824, CAS 330-55-2, Chem Services lot number 273-81B, expiration date 11/05 and CF-2006, CAS 330-55-2, Chem Services lot number 301-91A, expiration date 4/07. Stability testing was conducted with CF-1824 and in-life test solutions were made with CF-2006. Both chromatograms, after solvent blank correction, showed one large peak that had the appropriate retention time for linuron and several very small peaks. The area of the linuron peak was 99.6% for CF-1824 and 99.9% for CF-2006 of the total area of all peaks in the chromatograms. Chemical purity of linuron determined by the manufacturer was 99% for both lots (Appendix A).

3.2 Analytical Chemistry for Stability Testing

Chemical stability testing was initiated on 3/21/03. Chemical concentration was determined 11 times over a period of 19 days. The analytical and quality control (QC) results are presented in Appendix C. A preparation blank was analyzed with every sample batch for QC purposes. There were no detectable concentrations of linuron in the blanks except for one which was observed at less than five times the detection limit (DL). CCV results ranged from 99% to 104%. All QC data were within acceptance criteria. The DL was determined to be 123,950 ng/mL or 124 μg/mL.

Samples taken from the top and bottom of an ashed, amber bottle to evaluate the homogeneity of a 20 mg/mL concentration after stirring overnight had recoveries ranging from 92% to 102%. The relative standardized difference (RSD) for the samples taken from the top and bottom of the container was 5.5% and 5.3% respectively.

3.3 Statistical Results of Stability Trial

A plot of linuron with a target concentration of 5,000,000 ng/mL (or 5 mg/mL) against time suggests very little chemical loss (Figure 2). Only two data points were less than 90% of the target concentration. Based on the final regression model and the lower 95% confidence limit of the slope, the concentration of linuron was expected to stay greater than or equal to 90% of the target concentration for up to an estimated 6.5 weeks (Table 3). Thus, this stock solution was considered stable for the required 3-week holding period. The complete statistical analysis is presented in Appendix D.

3.4 Chemistry Results for the Analysis of In-Life Samples

In-life linuron concentrations had a mean of 7.19 and 15.4 mg/mL for the 10 and 15 mg/mL targets, respectively. Recoveries relative to the dosing target ranged from 31% to 130% for the 10 mg/mL target concentration and 40% to 174% for the 15 mg/mL target concentration during the course of the assay. The complete analysis is presented in Appendix E.

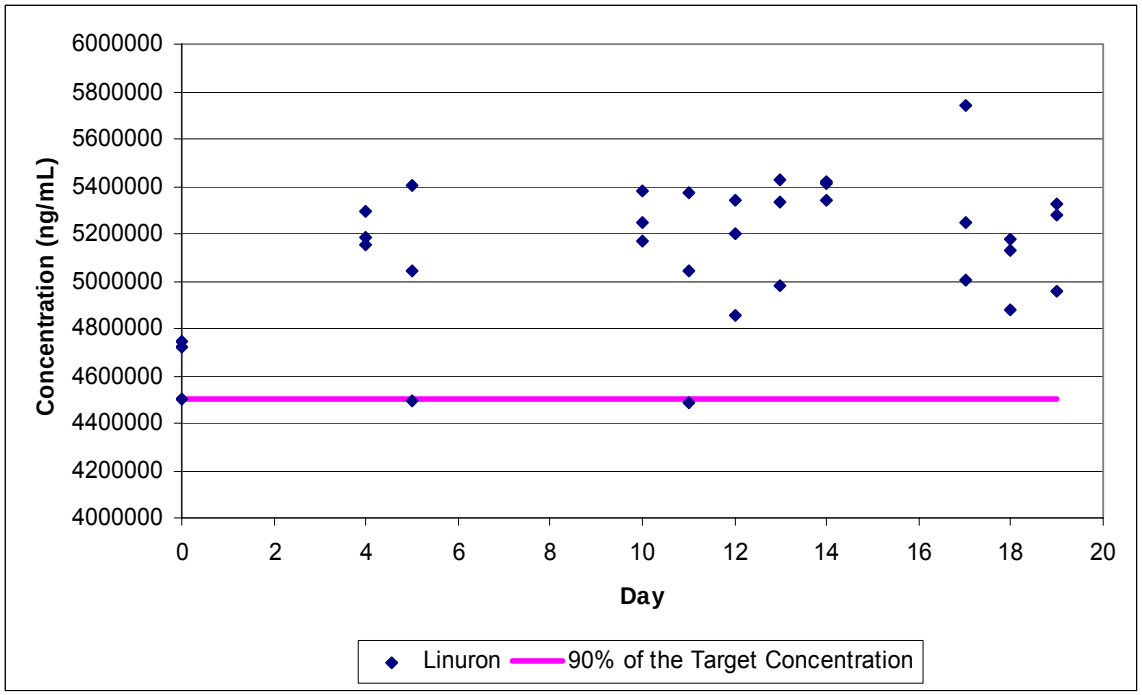


Figure 2. Observed concentration of linuron with a target concentration of 5,000,000 ng/mL against time

Table 3. Summary of Statistical Results for Linuron

WA 2-28-03-01 Statistical Analysis conducted by Valerie Cullinan Using Minitab Version 13.32, Minitab Inc., 1999.	1824-2a-3 Linuron
Target Concentration (ng/mL)	5,000,000
Number of determinations	1
Number of days tested	19
Number of replicates per day	3
Number of outliers removed	0
Number of observations removed	3
Overall Mean Concentration	5129649
95% Upper CL	5217348
error degrees of freedom	32
1-sample t-test of Ho: μ >= Target	NS [a]
estimated intercept of ln(concentration) against time	15.4395
estimated slope of ln(concentration) against time	0.0015
standard error of slope	0.0020
error degrees of freedom	28
Significance test of lack-of-fit for final model	NS
Significance test of Ho: $\beta = 0$ vs. H1: $\beta \neq 0$	NS
Lower 95% CL of β	-0.003
Upper 95% CL of β	0.006
Maximum Percent Loss (using LCL)	2.1%
Mean Percent Loss (using bhat)	-1.2%
LN(90% of Target)	15.3196
Number of days until at 90% of Target (using LCL)	46
Conclusion using Target Concentration:	**Stable for 21 days**

[a] Not Significant at $\alpha = 0.05$

4.0 CONCLUSIONS

The chemical purity determined by Battelle-Sequim for two lots of linuron both purchased from Chem Service was 99.6% for CF-1824 used in stability testing and 99.9% for CF-2006 used for in-life test solution preparation. The chemical purity of both lots as determined by the manufacturer was 99%. The homogeneity of a 20 mg/mL sample after stirring overnight was acceptable with percent recoveries ranging from 92% to 102%. Stability testing of linuron in methylcellulose concluded that the chemical was stable at 5 mg/mL for a holding period of 3 weeks.

In-life linuron concentrations had a mean of 7.19 and 15.4 mg/mL for the 10 and 15 mg/mL targets, respectively. Recoveries relative to the dosing target ranged from 31% to 130% for the 10 mg/mL target concentration and 40% to 174% for the 15 mg/mL target concentration during the course of the assay.

APPENDIX A

MANUFACTURER'S CERTIFICATES OF ANALYSIS/PURITY

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS237091
PO #: 11180291EAC

CATALOG #: PS-372 CAS #: 330-55-2

DESCRIPTION: Linuron

LOT #: 273-81B

PURITY: 99%

EXPIRATION DATE: 11/05

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, FTIR, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS238527
PO #: 11190692

CATALOG #: PS-372 CAS #: 330-55-2

DESCRIPTION: Linuron

LOT #: 301-91A

PURITY: 99%

EXPIRATION DATE: 04/07

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {062K0144} RESULTS
Product Name	Methylcellulose	
Product Number	M0512	
CAS Number	9004675	
APPEARANCE	WHITE WITH A YELLOW CAST POWDER	CONFORMS
SOLUBILITY	CLEAR TO HAZY COLORLESS TO LIGHT YELLOW VISCOUS SOLUTION AT 100 MG PLUS 5 ML OF WATER	VERY SLIGHTLY HAZY VERY FAINT YELLOW
VISCOSITY OF A 2% AQUEOUS SOLUTION	3500 TO 5600 CPS (20 DEG C)	4,202 CPS (SUPPLIER TEST RESULT)
QC ACCEPTANCE DATE		JULY 2002

David Feldker, Manager
Analytical Services

APPENDIX B

PURITY AND STABILITY TESTING PLAN

EDSP Purity Analysis and Stability Testing Plan for Linuron

Chemical Name: Linuron (MSL CF Login 1824)

CAS Number: 330-55-2

Lot Number: 273-81B
stored at RT in MSL5, Rm 219

Expiration date: 11/05

Manufacturer's Purity Information: 99%

Manufacturer's Stability Information: stable

MSL Purity Results:
Purity (%) To be determined at MSL by GC-FID scan

MDL has not been determined.

Bioassay Information:

Study Director
Name: Dr. Julia George
Affiliation: RTI
Location: RTP, NC
Telephone number: 919-541-5862

Proposed Bioassay: WA 2-28

Test Chemical: Linuron

CAS: 330-55-2

Carrier(s): 0.25% methylcellulose (Sigma M0512) in DI water

Concentration: 5.0 mg/mL

In vitro or in *vivo tests*? *In vivo*

Organism to be tested: rat

Method of test solution administration: oral gavage

Planned/Proposed test duration: 2 weeks

Design of Stability Test: 5.0 mg/mL in glass at 4 deg. C in the dark for 14 days, sample every two days in triplicate and analyzed by GC.

Number of replicates: 3

Duration: 14 days, sampling every two days

Other factors:

Temperature regime(s): 4 deg. C
Test container type: glass
Light or dark: dark except when container is removed for sampling or handling
Other: none

Statistical testing: regression analysis of the slope for concentration versus time

Resulting records package:

Manufacturer's certificate of analysis or purity
MSDS
Records:

- date sample received;
- date(s) sample analyzed;
- sample matrix;
- electronic file identification codes (when applicable to identify instrument data files);
- data summary reports;
  - Chemical repository confirmatory test results of chemical identity and purity;
  - Chemical repository test results of lot-to-lot variation in chemical purity;
  - Chemical repository periodic assessment results of changes in purity of stock solutions and dilutions and generation of degradation products
- QC data reports;
- data qualifying flags; and
- dilution factor(s).

APPENDIX C

ANALYTICAL RESULTS OF STABILITY TESTING

Table C1. Linuron concentration in a Methylcellulose and Water Suspension (ng/mL)

Target Conc.	Sample ID	Date Sampled	Date Analyzed	Linuron	Average (ng/mL)	Recovery
5000000 ng/mL	1824-2a-3-1 R-1	3/21/2003	3/27/2003	4503674		
5000000 ng/mL	1824-2a-3-1 R-2	3/21/2003	3/27/2003	4746162	4656947	93.1%
5000000 ng/mL	1824-2a-3-1 R-3	3/21/2003	3/27/2003	4721007		
Blank	1824-2a-3 Control 1		3/27/2003	123950 U		
5000000 ng/mL	1824-2a-3-2 R1	3/25/2003	3/27/2003	5296135		
5000000 ng/mL	1824-2a-3-2 R2	3/25/2003	3/27/2003	5156264	5211260	104%
5000000 ng/mL	1824-2a-3-2 R3	3/25/2003	3/27/2003	5181382		
Blank	1824-2a-3 Control 2		3/27/2003	123950 U		
5000000 ng/mL	1824-2a-3-3 R1 redo	3/26/2003	4/10/2003	4494469		
5000000 ng/mL	1824-2a-3-3 R2	3/26/2003	4/10/2003	5045530	4980102	99.6%
5000000 ng/mL	1824-2a-3-3 R3	3/26/2003	4/10/2003	5400307		
Blank	1824-2a-3 Control 3		4/10/2003	123950 U		
5000000 ng/mL	1824-2a-3-4 R1	3/31/2003	4/10/2003	5382523		
5000000 ng/mL	1824-2a-3-4 R2	3/31/2003	4/10/2003	5168065	5267032	105%
5000000 ng/mL	1824-2a-3-4 R3	3/31/2003	4/10/2003	5250509		
Blank	1824-2a-3 Control 4		4/10/2003	123950 U		
5000000 ng/mL	1824-2a-3-5 R1	4/1/2003	4/10/2003	5370104		
5000000 ng/mL	1824-2a-3-5 R2	4/1/2003	4/10/2003	4484112	4965296	99.3%
5000000 ng/mL	1824-2a-3-5 R3	4/1/2003	4/10/2003	5041672		
Blank	1824-2a-3 Control 5		4/10/2003	123950 U		
5000000 ng/mL	1824-2a-3-6 R1	4/2/2003	4/10/2003	5199018		
5000000 ng/mL	1824-2a-3-6 R2	4/2/2003	4/10/2003	5340336	5130404	103%
5000000 ng/mL	1824-2a-3-6 R3	4/2/2003	4/10/2003	4851857		
Blank	1824-2a-3 Control 6		4/10/2003	123950 U		
5000000 ng/mL	1824-2a-3-7 R1	4/3/2003	4/9/2003	4979836		
5000000 ng/mL	1824-2a-3-7 R2	4/3/2003	4/9/2003	5425226	5246516	105%
5000000 ng/mL	1824-2a-3-7 R3	4/3/2003	4/9/2003	5334488		
Blank	1824-2a-3 Control 7		4/9/2003	123950 U		
Blank	1824-2a-3 Control11		4/9/2003	241470.5		
5000000 ng/mL	1824-2a-3-8 R1	4/4/2003	4/9/2003	5340939		
5000000 ng/mL	1824-2a-3-8 R2	4/4/2003	4/9/2003	5414697	5390978	108%
5000000 ng/mL	1824-2a-3-8 R3	4/4/2003	4/9/2003	5417299		
Blank	1824-2a-3 Control 8		4/9/2003	123950 U		
5000000 ng/mL	1824-2a-3-9 R1	4/7/2003	4/9/2003	5000620		
5000000 ng/mL	1824-2a-3-9 R2	4/7/2003	4/9/2003	5249164	5329707	107%
5000000 ng/mL	1824-2a-3-9 R3	4/7/2003	4/9/2003	5739336		
Blank	1824-2a-3 Control 9		4/9/2003	123950 U		
5000000 ng/mL	1824-2a-3-10 R1	4/8/2003	4/9/2003	4875579		
5000000 ng/mL	1824-2a-3-10 R2	4/8/2003	4/9/2003	5176421	5059374	101%
5000000 ng/mL	1824-2a-3-10 R3	4/8/2003	4/9/2003	5126123		
Blank	1824-2a-3 Control10		4/9/2003	123950 U		
5000000 ng/mL	1824-2a-3-11 R1	4/9/2003	4/10/2003	4957557		
5000000 ng/mL	1824-2a-3-11 R2	4/9/2003	4/10/2003	5325908	5188525	104%
5000000 ng/mL	1824-2a-3-11 R3	4/9/2003	4/10/2003	5282109		
Blank	1824-2a-3 Control 11 redo		4/10/2003	123950 U		
			4/10/2003	123950 U		

Calculations of the average concentration and percentage recovery were made before rounding.

Table C.2. CCV Data for Linuron Concentration in a Methylcellulose and Water Suspension

Analysis Date	Sample Name	Linuron (ng/mL)	Recovery	PD
3/27/03	PP1192C500 ng/mLCCV	496.71	99.3%	0.66%
3/27/03	PP1192C500 ng/mLCCV	503.47	101%	0.69%
4/9/03	PP1192C500 ng/mLCCV	520.65	104%	4.13%
4/9/03	PP-1192 C500 ng/mLCCV	514.71	103%	2.94%
4/9/03	PP-1192C500 ng/mLCCV	513.77	103%	2.75%
4/10/03	PP-1192C500 ng/mLCCV	513.26	103%	2.65%
4/10/03	PP-1192C500 ng/mLCCV	508.89	102%	1.78%
4/10/03	PP-1192C500 ng/mLCCV	509.51	102%	1.90%
4/10/03	PP-1192C500 ng/mLCCV	509.56	102%	1.91%
5/6/03	PP-1192C500 ng/mLCCV	492.78	98.6%	1.44%

Calculations were made before rounding.

Table C.3. Homogeneity Data for Linuron in a Methylcellulose and Water Suspension

Expected Conc. (ng/mL)	Sample ID	Date Analyzed	Linuron	Average	RSD	Recovery
20000000	Top 1	5/6/03	20132028			101%
20000000	Top 2	5/6/03	20257586	19569846	5.54%	101%
20000000	Top 3	5/6/03	18319924			91.6%
20000000	Bottom 1	5/6/03	20444536			102%
20000000	Bottom 2	5/6/03	18628172	19268664	5.29%	93.1%
20000000	Bottom 3	5/6/03	18733284			93.7%

Calculations were made before rounding.

APPENDIX D

STATISTICAL REPORT

WA-2-28-03-01
Statistical Analysis conducted by Valerie Cullinan
Using Minitab Version 13.32, Minitab Inc., 1999.

8/6/03 12:23:28 PM

Analysis of linuron-5000000 in methylcellulose

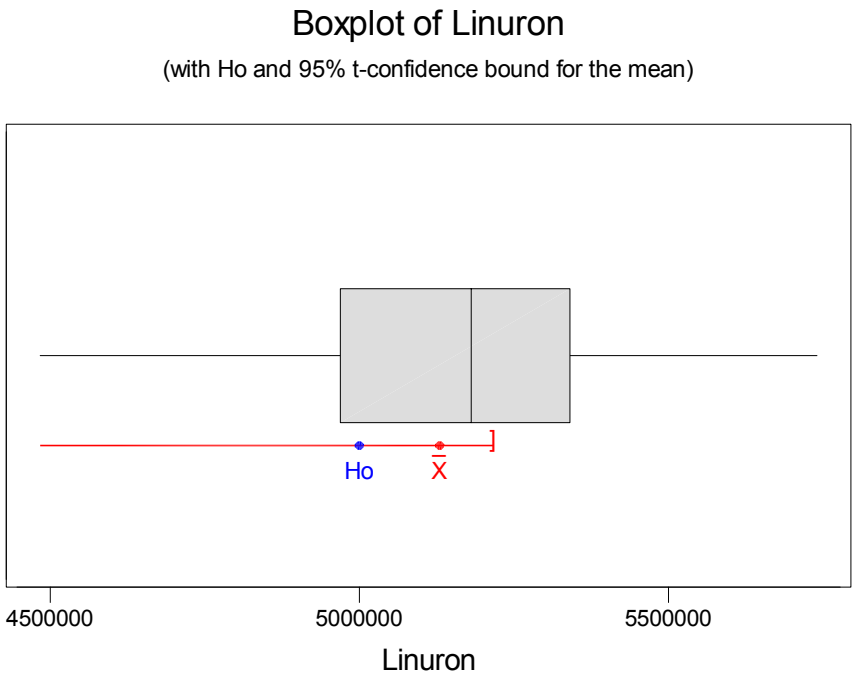
- Test to determine if the data are from a population with mean of 5000000.

```
Macro performs a one-sample t-test for mu less than TARGET &
What is the target value for X    3
DATA> 5000000
```

One-Sample T: Linuron

```
Test of mu = 5000000 vs mu < 5000000

Variable          N      Mean     StDev   SE Mean
Linuron          33   5129649    297418     51774

Variable      95.0% Upper Bound        T      P
Linuron                5217348     2.50  0.991      NS
```

t Boxplot of Linuron




```
Outliers are < Median-3*IQD OR > Median+3*IQD

Boundary for outliers are values <     4065558  and >     6297206

No outliers
```

- Transform data to natural logarithm and conduct regression analysis.

Week	Rep	Ln(Concentration)
0	1	15.3204
0	2	15.3728
0	3	15.3675
4	1	15.4825
4	2	15.4557
4	3	15.4606
5	1	15.3184
5	2	15.4340
5	3	15.5020
10	1	15.4987
10	2	15.4580
10	3	15.4738
11	1	15.4964
11	2	15.3161
11	3	15.4332
12	1	15.4640
12	2	15.4908
12	3	15.3949
13	1	15.4209
13	2	15.5066
13	3	15.4897
14	1	15.4909
14	2	15.5046
14	3	15.5051
17	1	15.4251
17	2	15.4736
17	3	15.5629
18	1	15.3997
18	2	15.4596
18	3	15.4499
19	1	15.4164
19	2	15.4881
19	3	15.4798

  - Conducts Simple Linear Regression

Regression Analysis: Linuron versus Day

The regression equation is
Linuron = 15.4 + 0.00413 Day

Predictor	Coef	SE Coef	T	P
Constant	15.4026	0.0208	741.00	0.000
Day	0.004134	0.001650	2.51	0.018

S = 0.05496 R-Sq = 16.8% R-Sq(adj) = 14.2%

Analysis of Variance

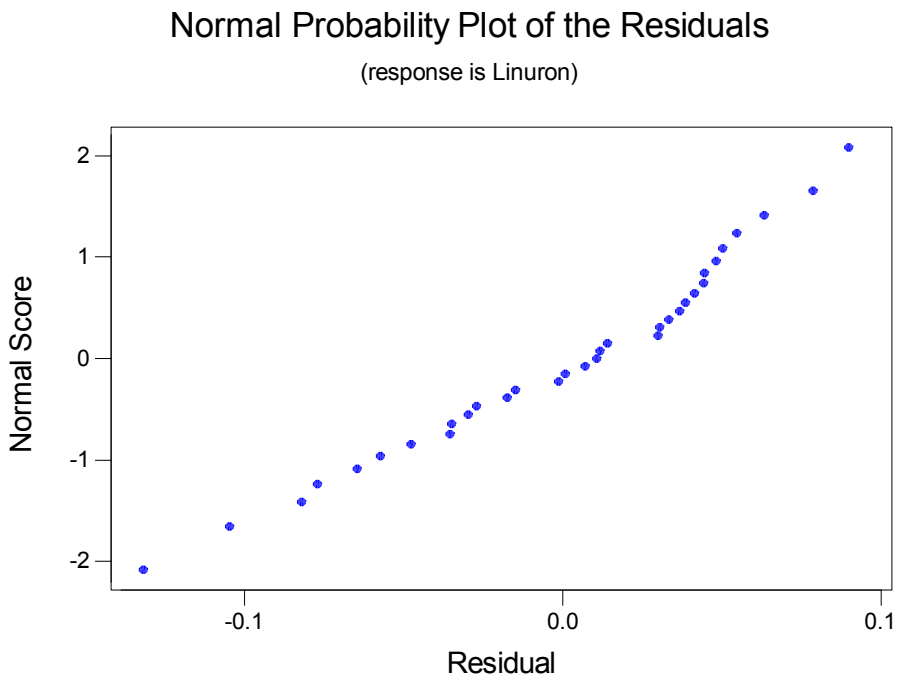
Source	DF	SS	MS	F	P	
Regression	1	0.018954	0.018954	6.28	0.018	
Residual Error	31	0.093628	0.003020			
Lack of Fit	9	0.032681	0.003631	1.31	0.287	NS
Pure Error	22	0.060947	0.002770			
Total	32	0.112582				

Unusual Observations

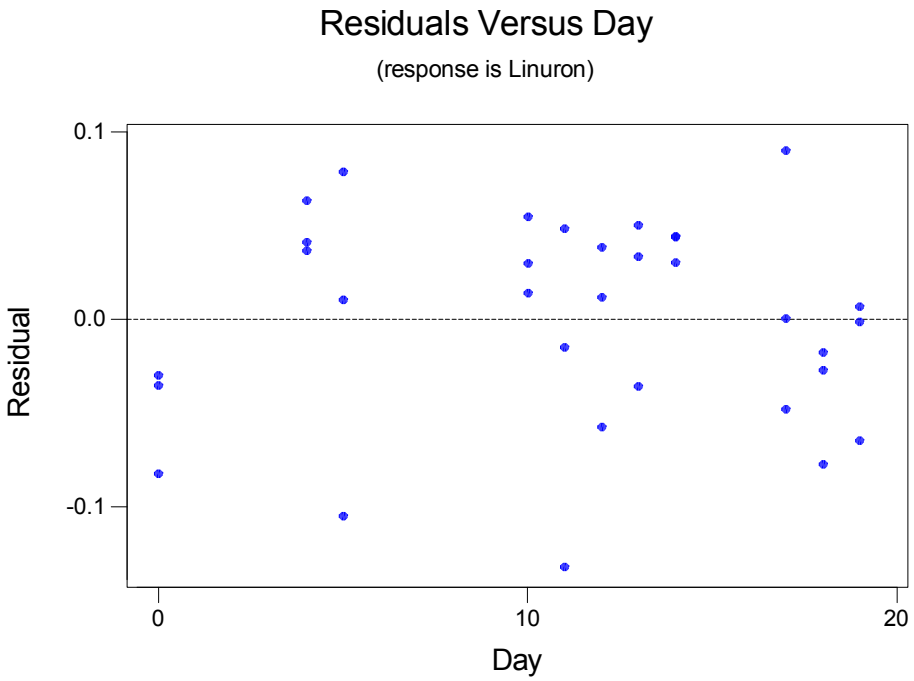
Obs	Day	Linuron	Fit	SE Fit	Residual	St Resid
14	11.0	15.3161	15.4481	0.0096	-0.1321	-2.44R

R denotes an observation with a large standardized residual

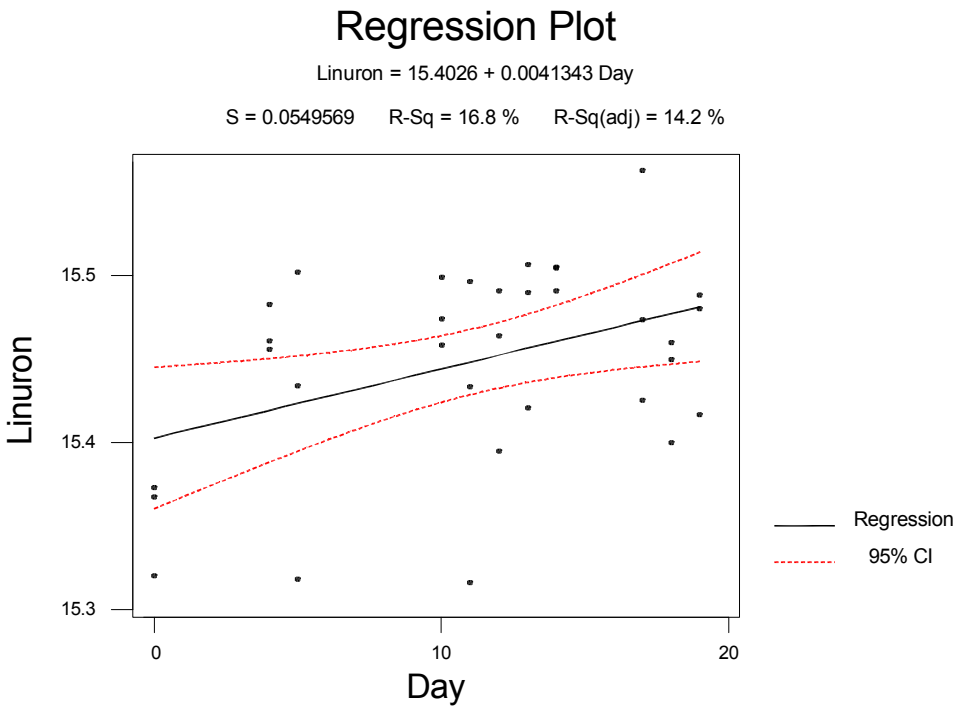
Normplot of Residuals for Linuron



Residuals from Linuron vs Day



Fitted Line Plot: Linuron versus Day



Re-do regression without day 0 data

———— 8/6/03 12:23:28 PM ————————————————————————

Regression Analysis: Linuron versus Day

The regression equation is
Linuron = 15.4 + 0.00154 Day
30 cases used 3 cases contain missing values

Predictor	Coef	SE Coef	T	P	
Constant	15.4395	0.0266	579.46	0.000	
Day	0.001535	0.002017	0.76	0.453	NS

S = 0.05322 R-Sq = 2.0% R-Sq(adj) = 0.0%

Analysis of Variance

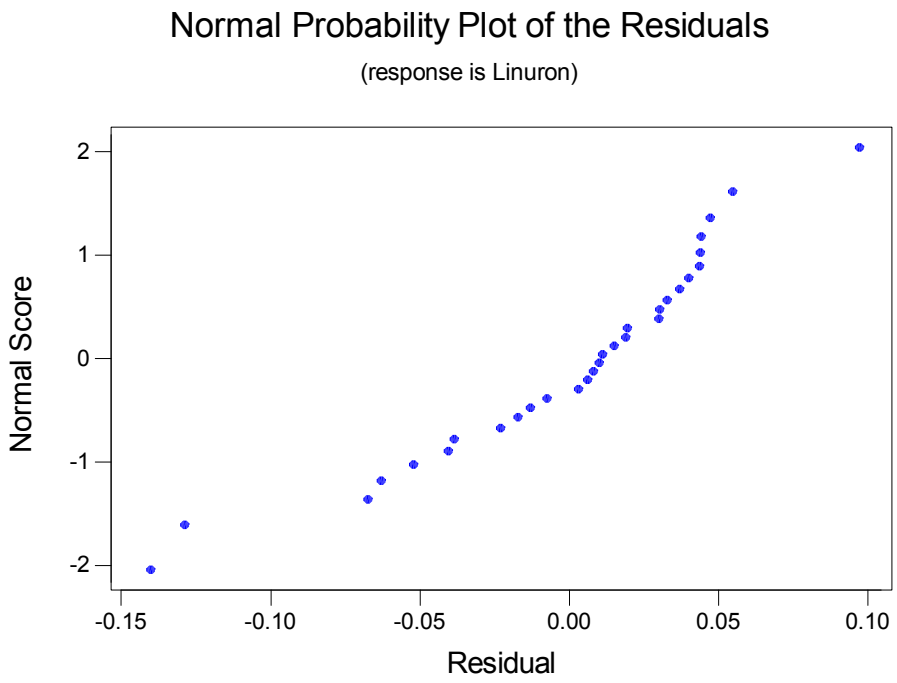
Source	DF	SS	MS	F	P	
Regression	1	0.001641	0.001641	0.58	0.453	
Residual Error	28	0.079320	0.002833			
Lack of Fit	8	0.020039	0.002505	0.85	0.575	NS
Pure Error	20	0.059280	0.002964			
Total	29	0.080961				

Unusual Observations

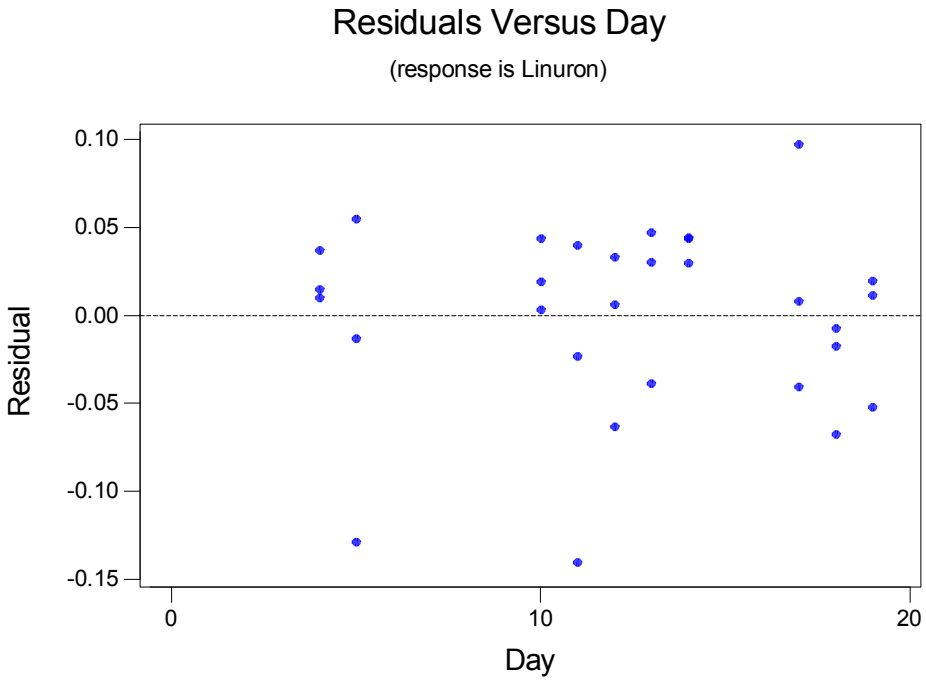
Obs	Day	Linuron	Fit	SE Fit	Residual	St Resid	
1	0.0	*	15.4395	0.0266	*	*	X
2	0.0	*	15.4395	0.0266	*	*	X
3	0.0	*	15.4395	0.0266	*	*	X
7	5.0	15.3184	15.4472	0.0176	-0.1288	-2.57R	
14	11.0	15.3161	15.4564	0.0101	-0.1403	-2.69R	

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

Normplot of Residuals for Linuron



Residuals from Linuron vs Day



- Power analysis for t-test of slope less than zero

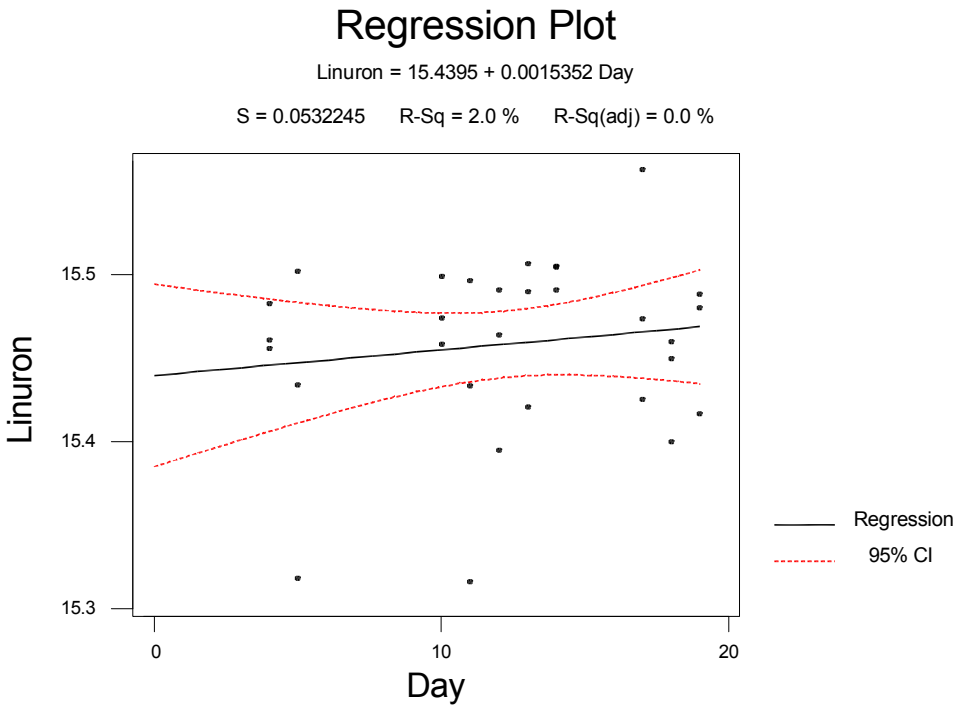
Power and Sample Size

```
1-Sample t Test

Testing mean = null (versus < null)
Calculating power for mean = null + difference
Alpha = 0.05  Sigma = 0.05322

Sample
  Size   Power  Difference
    28  0.9900     -0.0410
```

- That means we would detect a mean of 15.384 as significantly less than ln(5000000) = 15.425 or a change of 4799146 from 5000000 = 4.0% loss.

- Fit 95% confidence bands about the fitted simple linear model

Fitted Line Plot: Linuron versus Day



- **Conclusion – stable for 21 days.**

APPENDIX E

CHEMISTRY RESULTS
FOR THE ANALYSIS OF IN-LIFE SAMPLES

QA/QC Narrative

PROJECT:	**EDSP WA 2-28**
PARAMETER:	Linuron in a methyl cellulose and water suspension in-life test solution samples
LABORATORY:	Battelle Marine Sciences Laboratory 1529 West Sequim Bay Rd. Sequim, WA 98382
MATRIX:	Linuron in a methyl cellulose and water suspension
TEST SOLUTION SAMPLE CUSTODY AND PROCESSING:	Battelle, Sequim did not prepare the test solutions used in the WA 2-28 study. However, about 50 g of the test chemical linuron (CF 2006, Chem Service lot #301-91A, exp. 4/07) was sieved through a 500 um screen and shipped to RTI. 50 g of methyl cellulose (CF 1969, CAS 9004-67-5, Sigma Aldrich lot # 062K0144, assigned expiration date 1/2007) was also shipped to RTI for use in test solution preparation. Test solutions were prepared by RTI. The purity analysis was conducted on two lots of linuron; CF 1824 -1, CAS 330-55-2, Chem Service lot #273-81B, expiration date 11/2005 and CF 2006, CAS 330-55-2, Chem Service lot #301-91A, expiration date 4/2007. Stability testing was conducted with CF 1824 and in-life test solutions were made with CF 2006. Six (6) samples to evaluate homogeneity at two concentrations (5.0 mg/mL and 20.0 mg/mL) were returned from RTI on 5/19/03 and received at MSL on 5/20/03. Three samples labeled “dosage analysis” for concentrations at 0, 10.0 and 15.0 mg/mL were also returned with the sample set received on 5/20/03. Five (5) samples in a methyl cellulose matrix labeled 10303-93-11 to 10303-93-15 were shipped from RTI on 5/27/03 and received on 5/28/03 at 4.4 ^{o}C. These were given Battelle code 2006-11-15. Five (5) samples in a methyl cellulose matrix labeled 10303-93-11 to 10303-93-15 were shipped from RTI on 6/2/03 and received on 6/3/03 at 1.9 ^{o}C. There were two samples listed as 10303-93-14 on RTIs Chain of Custody form, but the sample receipt staff noted that on the container labels one sample was labeled 10303-93-14 and another was labeled 10303-93-15. These were given Battelle code 2006-16-20. In the case of the two sample sets of five with the same sample codes; RTI must be advised to assign unique codes to each sample. Twenty-four (24) post-dosing samples (21a&b through 32a&b) were shipped from RTI on 6/9/03 and received on 6/10/03 at 2.6 ^{o}C. RTI’s labeling was maintained through sample log-in analysis and reporting (Tables E1 and E2).

SAMPLE PREPARATION AND ANALYSIS	Samples were received in 20 mL scintillation vials and were prepared for analysis by weighing the vials before samples were removed for analysis. 10 mL acetonitrile (ACN, JT Baker lot # Y02820) was pipetted into each vial, agitated and then poured into a 30 mL amber glass vial. Then 15 mL of ACN was added to rinse the scintillation vial bring the total volume to 25 mL. The scintillation vial was air dried and then weighed to determine a tare weight from which the sample weight could be calculated. The 25 mL ACN extracts were prepared by taking 10 uL (lower concentrations and control) or 5 uL (two higher concentrations; 15 and 20 mg/mL) of extract to 0.99 mL of 60% ACN (40% water). This solution was then analyzed by high performance liquid chromatography (HPLC) using an ultraviolet/visible wavelength (UV/VIS) detector at the 250 nm wavelength with a 60% isocratic ACN eluent. Separation was attained using a Phenomenex Hydro C-18 column. Samples were analyzed 5/21/03 and 6/11/03. Calibration was performed on 5/21/03 and 6/11/03 using dilutions prepared from the standard PP-1192 A thru E at 50, 200, 500, 2000, and 5000 ng/mL. HPLC runs are stored on the computer with the property number WV04738. For blanks and low concentration samples (5 and 10 mg/mL) , 0.01 mL of the sample is pipetted into a 1.8 mL vial with 0.99 mL of a 60% ACN/water solution. For higher concentration samples (15 and 20 mg/mL) 0.005 mL of sample and 0.995 mL 60% ACN/water solution are combined for analysis. 250 uL samples are injected for HPLC analysis. The twenty-four (24) post-dosing samples (21a&b through 32a&b) were processed as described above for 10 and 15 mg/mL concentrations. Samples were 0.005 mL.
TEST SOLUTION SAMPLE ANALYSIS	Sample solutions were analyzed by HPLC using an ultraviolet/visible wavelength (UV/VIS) detector at the 250 nm wavelength with a 60% isocratic ACN eluent. Separation was attained using a Phenomenex Hydro C-18 column. Samples were analyzed on 5/21/03 and 6/11/03. Calibration was performed on 5/21/03 and 6/11/03 using dilutions prepared from the standard PP-1192 at 50, 200, 500, 2000, and 5000 ng/mL. HPLC runs are stored on the computer with the property number WV04738.

Data Quality Objectives:	Control Limits
Procedural Blank	< 5 * MDL
Continuing Standard Recovery	75-125%

IN-LIFE QC DATA SUMMARY

METHODS:	HPLC using a UV/VIS wavelength detector at the 250 nm wavelength with a 60% isocratic ACN eluent. Separation was attained using a Phenomenex Hydro C-18 column.
CALIBRATION:	Samples were analyzed on 5/21/03 and 6/11/03. Calibration was performed on 3/27/03 using dilutions prepared from the standard PP-1192 at 50, 200, 500, 2000, and 5000 ng/mL. Calibration was again performed on 6/09/03 using dilutions prepared from the standard PP-1192 at 50, 200, 500, 2000, and 5000 ng/mL.
CONTINUING STANDARD RECOVERY:	Six CCVs were analyzed with the in-life samples. A mean of 101 percent recovery was attained with a range between 100 to 103%.There were unacceptable CCVs analyzed that resulted in re-runs of samples as the required corrective action. The six reported CCVs are from successful runs.
BLANK	Three blanks were analyzed in the successful runs and all were below the MDL.
INTERNAL STANDARD	No internal or surrogate standards were analyzed with linuron samples.
DETECTION LIMIT:	The DL determined from analysis of the low standard (49.59 ug/L) was 123950 ng/mL.
HOLDING TIME:	The times of sample collection, receipt, and analysis for the in-life data are provided in the following tables. All samples were received and analyzed within 15 days of receipt. Samples were deemed to be stable for 21 days. Some samples were analyzed after the stated holding time.

Table E1. In-life data from sample formulations prepared by and provided to Battelle, Sequim by RTI.

MSL Sample ID	RTI Sample ID	Sample Description	Target Concentration (mg/mL)	Linuron (mg/mL)	Percent Recovery Based on Target	Prep Date	Collection Date	Received At MSL	Analyzed
2006-2	10303-89-15	Dosage Analysis	0	0.12 U[a]	--	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-6	10303-89-19	Dosage Analysis	10.0	9.97	100%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-7	10303-89-20	Dosage Analysis	15.0	14.2	95%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-3	10303-89-16	Homogeneity-top	5.0	5.07	101%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-4	10303-89-17	Homogeneity-middle	5.0	5.30	106%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-5	10303-89-18	Homogeneity-bottom	5.0	3.46	69%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
			Mean	4.61	92%				
			CV	21.7%					
2006-8	10303-89-21	Homogeneity-top	20.0	20.1	101%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-9	10303-89-22	Homogeneity-middle	20.0	16.2	81%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2006-10	10303-89-23	Homogeneity-bottom	20.0	19.5	98%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
			Mean	18.6	93%				
			CV	11.3%					
2006-11	10303-91-11	0.25 % MC	0	0.12 U	--	5/23/2003	5/23/2003	5/28/2003	6/11/2003
2006-12	10303-91-12	0.25 % MC	5.0	3.53	71%	5/23/2003	5/23/2003	5/28/2003	6/11/2003
2006-13	10303-91-13	0.25 % MC	10.0	8.09	81%	5/23/2003	5/23/2003	5/28/2003	6/11/2003
2006-14	10303-91-14	0.25 % MC	15.0	11.8	71%	5/23/2003	5/23/2003	5/28/2003	6/11/2003
2006-15	10303-91-15	0.25 % MC	20.0	16.6	83%	5/23/2003	5/23/2003	5/28/2003	6/11/2003
2006-16	10303-93-11	0.25 % MC	0	0.12 U	--	5/30/2003	5/30/2003	6/3/2003	6/11/2003
2006-17	10303-93-12	0.25 % MC	5.0	6.13	123%	5/30/2003	5/30/2003	6/3/2003	6/11/2003
2006-18	10303-93-13	0.25 % MC	10.0	8.48	85%	5/30/2003	5/30/2003	6/3/2003	6/11/2003
2006-19	10303-93-14	0.25 % MC	15.0	16.2	108%	5/30/2003	5/30/2003	6/3/2003	6/11/2003
2006-20	10303-93-15	0.25 % MC	20.0	14.2	71%	5/30/2003	5/30/2003	6/3/2003	6/11/2003

[a] Undetected at reported method detection limit, 0.12 mg/mL

Calculations were conducted before rounding.

Table E2. In-life data from sample formulations collected at intervals over the course of the study

MSL Sample ID	RTI Sample ID	Sample Description	Target Concentration (mg/mL)	Linuron (mg/mL)	Percent Recovery Based on Target	Prep Date	Collection Date	Received At MSL	Analyzed
2006-21a	10303-89-03	First Day of Dosing Sample	10	6.63	66%	5/17/2003	5/20/2003	6/10/2003	6/11/2003
2006-21b	10303-89-03	First Day of Dosing Sample	10	6.91	69%	5/17/2003	5/20/2003	6/10/2003	6/11/2003
2006-23a	10303-89-03	Last Day of Dosing Sample	10	8.63	86%	5/17/2003	5/25/2003	6/10/2003	6/11/2003
2006-23b	10303-89-03	Last Day of Dosing Sample	10	9.96	100%	5/17/2003	5/25/2003	6/10/2003	6/11/2003
2006-25a	10303-91-03	First Day of Dosing Sample	10	4.59	46%	5/23/2003	5/26/2003	6/10/2003	6/11/2003
2006-25b	10303-91-03	First Day of Dosing Sample	10	5.38	54%	5/23/2003	5/26/2003	6/10/2003	6/11/2003
2006-27a	10303-91-03	Last Day of Dosing Sample	10	3.11	31%	5/23/2003	6/1/2003	6/10/2003	6/11/2003
2006-27b	10303-91-03	Last Day of Dosing Sample	10	4.61	46%	5/23/2003	6/1/2003	6/10/2003	6/11/2003
2006-29a	10303-93-03	First Day of Dosing Sample	10	7.28	73%	5/30/2003	6/2/2003	6/10/2003	6/11/2003
2006-29b	10303-93-03	First Day of Dosing Sample	10	13.0	130%	5/30/2003	6/2/2003	6/10/2003	6/11/2003
2006-31a	10303-93-03	Last Day of Dosing Sample	10	7.84	78%	5/30/2003	6/5/2003	6/10/2003	6/11/2003
2006-31b	10303-93-03	Last Day of Dosing Sample	10	8.42	84%	5/30/2003	6/5/2003	6/10/2003	6/11/2003
2006-22a	10303-89-04	First Day of Dosing Sample	15	14.2	95%	5/17/2003	5/20/2003	6/10/2003	6/11/2003
2006-22b	10303-89-04	First Day of Dosing Sample	15	13.3	89%	5/17/2003	5/20/2003	6/10/2003	6/11/2003
2006-24a	10303-89-04	Last Day of Dosing Sample	15	24.3	162%	5/17/2003	5/25/2003	6/10/2003	6/11/2003
2006-24b	10303-89-04	Last Day of Dosing Sample	15	23.8	159%	5/17/2003	5/25/2003	6/10/2003	6/11/2003
2006-26a	10303-91-04	First Day of Dosing Sample	15	7.43	50%	5/23/2003	5/26/2003	6/10/2003	6/11/2003
2006-26b	10303-91-04	First Day of Dosing Sample	15	6.59	44%	5/23/2003	5/26/2003	6/10/2003	6/11/2003
2006-28a	10303-91-04	Last Day of Dosing Sample	15	7.86	52%	5/23/2003	6/1/2003	6/10/2003	6/11/2003
2006-28b	10303-91-04	Last Day of Dosing Sample	15	6.02	40%	5/23/2003	6/1/2003	6/10/2003	6/11/2003
2006-30a	10303-93-04	First Day of Dosing Sample	15	17.1	114%	5/30/2003	6/2/2003	6/10/2003	6/11/2003
2006-30b	10303-93-04	First Day of Dosing Sample	15	15.1	101%	5/30/2003	6/2/2003	6/10/2003	6/11/2003
2006-32a	10303-93-04	Last Day of Dosing Sample	15	23.6	157%	5/30/2003	6/5/2003	6/10/2003	6/11/2003
2006-32b	10303-93-04	Last Day of Dosing Sample	15	26.1	174%	5/30/2003	6/5/2003	6/10/2003	6/11/2003

Battelle
Putting Technology To Work

Chemical Repository Services for the EDSP

EPA Contract No. 68-W-01-023

Chemistry Report for WA 2-28
Methoxychlor in Methylcellulose

Revised March 24, 2005

Prepared By:		Approved By:	
Eric A. Crecelius	3/24/05	RM Ecker	3/24/05
Eric A. Crecelius, Ph.D. Chemical Repository Manager	Date	Richard M. Ecker Director, Marine Sciences Laboratory	Date

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Submitted to:

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Research Triangle Institute

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Research Triangle Park, NC 27709

Chemistry Report for WA 2-28
Methoxychlor in Methylcellulose

Reviewed by: Mary E Lynn

Mary E Lynn, EDSP Quality Assurance Representative

Battelle Marine Sciences Laboratory

Date: 3/24/05

Chemistry Report for WA 2-28
Methoxychlor in Methylcellulose

Parameter	Chemical
Compound Name	Methoxychlor
CAS #	72-43-5
Central File No.	CF-1839
Initial Receipt Date	10/11/02
Expiration Date	6/1/05
Manufacturer	Sigma, Inc.
Lot Number	049H1328
Battelle Study #	WA 2-28-03-01
Method	SW 846, 8015B Modified

Executive Summary

The chemical purity of methoxychlor determined by the manufacturer was 95.2%. The purity result from Battelle-Sequim by GC-FID was determined to be 89.99%. Based on the final regression model and the lower 95% confidence limit of the slope, the concentration of methoxychlor was expected to stay greater than or equal to 90% of the Day 0 concentration for up to an estimated 16 days. Thus, stability testing of the methoxychlor stock solution in methylcellulose was considered stable at the average day 0 concentration of 2352 μg/mL for the required shipping and holding period of 2 weeks.

Methylcellulose (CF-1969; lot # 062K0144; and expiration date 3/07) was purchased 1/31/03 from Sigma, Inc. to be used as a carrier for the stability testing. The carrier had no visual defects and was stored at room temperature.

In-life chemical concentrations were determined for 24 samples collected during the 15 day period that the rats were dosed (solutions were not prepared at Battelle-Sequim; the samples were returned from Research Triangle Institute). Of the in-life samples that were analyzed for the target concentration of 5.0 mg/mL, the mean concentration measured was 5.26 mg/mL and the range of was 73-161% of the target. Of the in-life samples that were analyzed for the target concentration of 7.5 mg/mL, the mean concentration measured was 7.88 mg/mL and the range of recovery was 49-139% of the target.

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1.0 INTRODUCTION

The goal of the Battelle-Sequim, Marine Sciences Laboratory (MSL) Chemical Repository for the Endocrine Disruptor Screening Program (EDSP) is to provide the participating laboratory with requested chemicals of documented quality at required concentrations and in a matrix appropriate for different toxicological tests. The EDSP Chemical Repository supplies the manufacturer's information regarding purity and stability, the material safety data sheet (MSDS) chemical information, and independent analysis of purity and stability in a matrix specified by the Purity and Stability Testing Plan made in collaboration with the requesting Principal Investigator. Additional analysis associated with the in-life studies are also provided when requested. This report is the product of such a request.

Under Work Assignment (WA) 2-28 and Battelle-Sequim Study Number WA 2-28-03-01, Dr. Julia George from Center for Life Sciences and Toxicology, Research Triangle Institute, requested purity and stability testing of methoxychlor (Figure 1). Electronic files submitted to the EDSP Data Coordination Center in support of this work assignment were CRF_WA-2-28_methoxychlor-mc.doc, PSTP_WA-2-28_methoxychlor-methylcellulose.doc, DSUM_WA-2-28_Methoxychlor-mc.xls, and DAF_WA-2-28_methoxychlor-mc.doc.

2.0 GENERAL METHODS

Methods of standard operation of the Chemical Repository are addressed in the procedure, EDSP.C-001-01, The EDSP Chemical Repository. This procedure addresses chemical procurement including procurement of controlled substances, when applicable, which have unique permitting, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage; stock solution preparation, documentation and archiving; test solution preparation, documentation and shipping; chemical disposal, and repository maintenance over time. The quality assurance (QA) requirements for procurement of chemicals for use in the Chemical Repository are addressed in procedure, MSL-A-012, Procurement. Each purchase requisition receives QA review to determine what is being ordered and which specific requirements apply.

2.1 Chemical Procurement

As requested by Dr. Julia George, methoxychlor (CAS No. 72-43-5) was purchased for purity and stability analysis for use in a bioassay on rats (Figure 1). Methoxychlor was purchased from Sigma, Inc. and lot number 049H1328 was received on 10/11/02 with an expiration date of 6/1/05 (Table 1). The chemical was left in the original container, logged in to the Chemical Management System (CMS) and given a CMS barcode and unique log in number (CF-1839) as per the QA Project Plan (QAPP) for the EDSP Chemical Repository. The chemical was stored in a dry location at room temperature, away from direct sunlight.

Methylcellulose (CF-1969; lot # 062K0144; and expiration date 3/07) was purchased 1/31/03 from Sigma, Inc. to be used as a carrier for the stability testing. The carrier had no visual defects and was stored at room temperature.

EDSP Chemical Request Form

For EPA WA: 2-28-03-01

Study Director

Name: Dr. Julia George
Affiliation: Center for Life Sciences and Toxicology
Research Triangle Institute
Location: PO Box 12194
Research Triangle Park, NC 27709
Telephone number: 919-541-5862

Bioassay Information

Proposed Bioassay: WA 2-28

Test Chemical: Methoxychlor (MSL CF 1839)

Carrier(s): Methylcellulose

Concentrations/Dilution Series: 2.5 mg/mL

*Consider if analysis method detection limit which may be determined in Purity analysis is above or below desired test concentrations?

In vitro or *in vivo* tests? *In vivo*

Organism to be tested: rat

Method of test solution administration: oral gavage

Planned/proposed test duration: 6 weeks

Chemical Information

Chemical Name: Methoxychlor

CAS: 72-43-5

Any known purity information: may refer to attached documentation

Manufacturer's Purity Information: 95.2% pure

Any known stability information: may refer to attached documentation

Manufacturer's Stability Information: stable

Desired purity (%) for test? 95% or greater

Figure 1. EDSP Requisition Form for Methoxychlor

Table 1. Chemical Procurement Information

Parameter	Chemical
Compound Name	Methoxychlor
CAS #	72-43-5
Central File No.	CF-1839
Initial Receipt Date	10/11/02
Expiration Date	6/1/05
Manufacturer	Sigma, Inc.
Lot Number	049H1328
Manufacturer's Purity	95.2%
Storage Conditions	Dry place/room temp.
Battelle Study #	WA 2-28-03-01
Method	SW 846, 8015B Modified

2.2 Chemical Purity

Chemical purity was verified by chromatographic analysis to determine areas under peaks other than the principal peak and compared to the manufacturer's certificate of analysis/purity (Appendix A). No statistical analyses were performed for the verification of chemical purity. General methods are documented in the procedure, EDSP.D-012-01, Chemical Repository Summary Displays and Statistical Analyses for the EDSP Data Coordination Center (DCC).

Purity verification was conducted by making a solution in hexane of about 100 µg/mL. This matrix was then analyzed on a gas chromatograph with a flame ionization detector (GC-FID). A hexane blank was also analyzed on the GC-FID. The purity was determined by first identifying the peaks in the chromatogram of the methoxychlor that were the same as the peaks in the analysis of the blank hexane sample. The areas associated with these common peaks were then eliminated by inhibiting integration and the remaining peaks were reported as a percentage of the total peak area. The percentage associated with the largest peak represented the purity of methoxychlor. The GC was set up with an autosampler and a 30 m x 0.25 mm, DB-5 capillary column. The temperature program was set to start at 50ºC, and ramped at 10ºC/min to a final temperature of 320ºC. The injection port temperature was set at 270ºC and the detector at 320ºC. The autosampler was set to inject 1 µL of the matrix dilution. One replicate was analyzed.

2.3 Preparation of Stock Matrices for Stability Analysis

A general study plan for stability testing based on the WA 2-28 request from Dr. Julia George was developed as the stability test protocol and is presented in Appendix B. A single stock solution was prepared to arrive at the chemical concentration requested for stability analysis (Table 2). The sample was analyzed in triplicate so that a mean concentration and relative standard deviation (RSD) could be determined. General methods are documented in EDSP.D-012-01.

Methoxychlor stock matrices were prepared at Battelle-Sequim on 4/22/03 for stability testing as described in Table 2. Briefly, the stock 0.25% methylcellulose suspension for stability testing was prepared by adding 1 g methylcellulose (CF 1969, lot # 062K0144, exp. 3/07) and 1 gram of methoxychlor (CF 1839, lot # 49H1328, exp. 6/1/05) which had been passed through a 80 mesh sieve into a tared 500 ml. amber bottle. House DI water was then added for a final total

weight of 400 grams. This solution was then stirred overnight using a stir bar and a magnetic stirrer. The container was labeled and stored at 4ºC±2ºC for the duration of the test.

Table 2. Stock Matrix Composition for Stability Testing

Study and Duration	Test Chemical	Target Concentration	Sample ID	Stock Matrix
WA 2-28-03-01 16 Days	Methoxychlor (95% purity)	2500 μg/mL	1839-2a-4	1.05 g in 400 mL of a 0.25% methylcellulose suspension in reagent water

2.4 Analytical Chemistry for Stability Testing

Chemical stability was evaluated under storage conditions and matrix specifications as requested by the participating laboratory. At initiation and at each time period throughout the duration of the test, the concentration was determined by chromatographic analysis. Triplicate aliquots were tested. The frequency of determinations and the duration of testing were determined by the requesting principal investigator and the chemists based on *a priori* knowledge about chemical stability. General methods are documented in EDSP.D-012-01.

The Methoxychlor stock solution was sampled by collecting 1 mL samples from the top, middle, and bottom of the container. Samples were placed into tared 25 mL amber vials, and the sample weight was determined gravimetrically. About 1.0 g sodium chloride (EM Science lot # 303OB06), and 25 mL methylene chloride (JT Baker Lot # 36E04) were added to the sample. Then 0.1 mL internal standard, PP-1215 (10000 μg/mL, 5a androstane) was added and the sample agitated for 2 minutes. Then, a 0.5 mL sample was added to 0.50 mL hexane (JT Baker, lot # 40E12) in a GC autosampler vial. Samples were analyzed using a GC-FID. Initial and continuing calibration verification (ICV/CCV) was accomplished using dilutions of analytical standard EDSP Mix 1. GC-FID runs are stored on the computer, WD35603, in room 223, MSL 5. Samples analyzed before 4/29/03 were quantitated against the 2/6/03 calibration. A full calibration check was analyzed on 4/25/03. Samples analyzed after 4/29/03 were quantitated against that calibration. A methylcellulose blank was prepared the same way. This solution was then run on the GC-FID for quantification.

2.5 Statistical Analysis of Stability

Following a test of homogeneity of the sections sampled, log linear degradation curves were fit to the data to describe the chemical concentration vs. time trends and their dependence on storage conditions and solvent matrix. Lack of fit and residual plots were evaluated to determine the form of the regression. Power calculations based on the observed variability were used to determine the sensitivity of the test to detect degraded concentrations. General methods are documented in SOP EDSP.D-012-01.

2.6 Analytical Chemistry for In-Life Testing

Analytical methods associated with in-life testing were similar to those described in Section 2.4. In-life analyses were conducted on samples returned from the bioassay laboratory, and included two sets of samples to assess homogeneity of test solutions, two samples to assess

dosage preparation at 5.0 and 7.5 mg/mL concentrations, and two sample sets to verify the range of test sample concentrations (2.5, 5.0, 7.5, and 10.0 mg/mL) during the course of the test. In-life samples at first and last dosing were also returned for analyses; there were no control samples returned.

For samples returned to Battelle in scintillation vials, the processing was as follows. First, the vial was weighed, and the contents poured into a 25 mL amber glass container. Then, a total of 25 mL methylene chloride (JT Baker Lot # 36E04) was used to remove any remaining sample from the scintillation vial. The methylene chloride was added in two aliquots: first, 10 mL was added to the scintillation vial and agitated; then the methylene chloride was added to the amber glass container. This was repeated with 15 mL methylene chloride, which was then also added to the amber glass container. The rinsed scintillation vials were allowed to dry, and the weight of the empty scintillation vials was determined so that the sample weight could be calculated from the difference of the two weights measured. After the above procedure, 2.0 g sodium chloride (EM Science lot # 303OB06) was added to the amber glass container, and this was shaken by hand for 2 minutes. Then 0.2 mL methylene chloride was removed from the amber glass container and pipetted into a 1.8 mL autosampler vial with 0.02 mL internal standard, PP-1219 (1004 μg/mL, 5a androstane) and 0.78 mL hexane (JT Baker, lot # 40E12). This was analyzed by GC-FID.

3.0 RESULTS

3.1 Chemical Purity

Battelle-Sequim ran a GC-FID purity scan on the methoxychlor. The chromatogram, after solvent blank correction, showed one large peak that had the appropriate retention time for methoxychlor and several very small peaks. The area of the methoxychlor peak was 89.99%of the total area of all peaks in the chromatogram. Chemical purity of methoxychlor determined by the manufacturer was 95.2% (Appendix A).

3.2 Analytical Chemistry for Stability Testing

Chemical stability testing was initiated on 4/22/03. Chemical concentration was determined 10 times over a period of 16 days. The analytical and QC results are presented in Appendix C. A single preparation blank was analyzed with every batch for quality control purposes. There were no detectable concentrations of methoxychlor in the blanks. ICV/CCV results ranged from 84.0% to 106%. Internal standards were not analyzed. The instrument detection limit (IDL) was 25 μg/mL which is the lowest calibration standard taking into account the dilution factor.

3.3 Statistical Results of Stability Trial

Homogeneity of the sections sampled (top, middle, and bottom) was not rejected ($p = 0.882$, $n = 29$) with the nonparametric Kruskal-Wallis test. A plot of methoxychlor with a target concentration of 2500 μg/mL against time showed all but three observations less than the target concentration (Figure 2). Thus, the average day 0 concentration of 2352 μg/mL was used to test stability. None of the data points were less than 90% of the day 0 concentration. Based on the final regression model and the lower 95% confidence limit of the slope, the concentration of methoxychlor was expected to stay greater than or equal to 90% of the target concentration for up to an estimated 16 days (Table 3). Thus, this stock solution was considered stable for the required 2-week holding period. The complete statistical analysis is presented in Appendix D.

3.4 Chemistry Results for the Analysis of In-Life Samples

Formulation samples and in-life samples were analyzed for chemical concentrations (solutions were not prepared at Battelle-Sequim; the samples were returned from Research Triangle Institute). The complete analysis is presented in Appendix E.

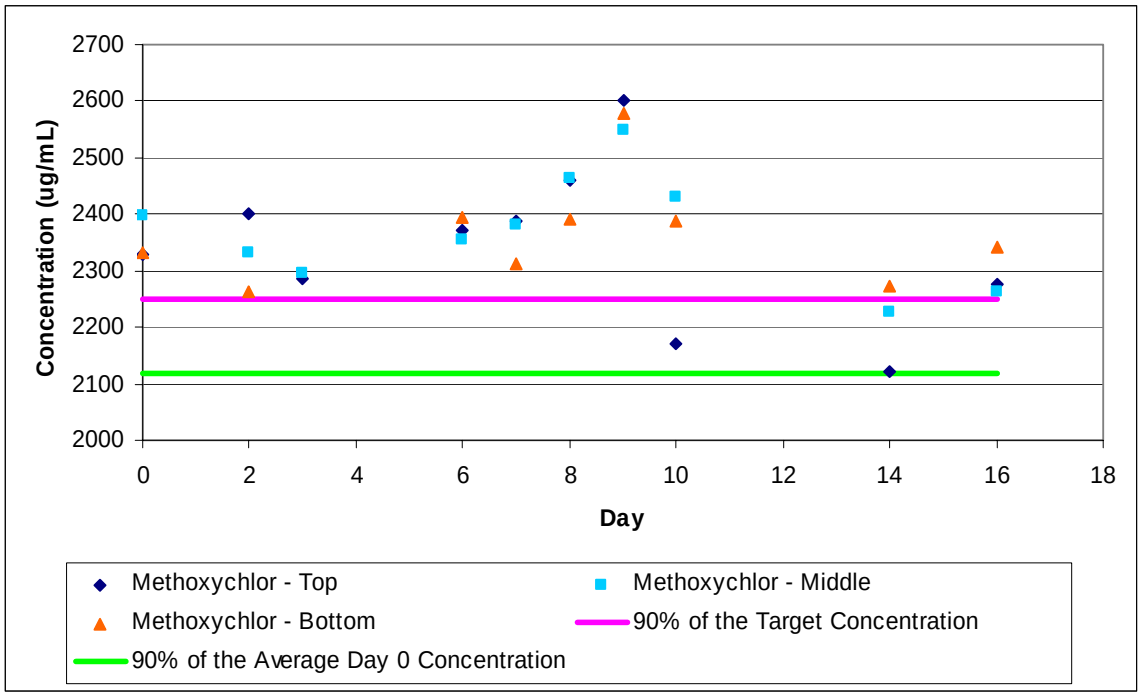


Figure 2. Observed concentration of methoxychlor with a target concentration of 2500 µg/mL and an average day 0 concentration of 2352 µg/mL against time

Table 3. Summary of Statistical Results for Methoxychlor

WA 2-28-03-01 Statistical Analysis conducted by Valerie Cullinan Using Minitab Version 13.32, Minitab Inc., 1999.	1839-2a-4 Methoxychlor
Target Concentration (μg/mL)	2500
Number of determinations	1
Number of days tested	16
Number of replicates per day assuming homogenous	3
Number of outliers removed	0
Number of observations removed	0
Overall Mean Concentration	2358
95% Upper CL	2392
error degrees of freedom	28
1-sample t-test of Ho: μ >= Target	S [a]
estimated intercept of ln(concentration) against time	7.7775
estimated slope of ln(concentration) against time	-0.0017
standard error of slope	0.0018
error degrees of freedom	27
Significance test of lack-of-fit for final model	S
Significance test of Ho: β = 0 vs. H1: β = 0	NS [b]
Lower 95% CL of β	-0.005
Upper 95% CL of β	0.002
Maximum Percent Loss (using LCL)	4.2%
Mean Percent Loss (using bhat)	1.4%
LN(90% of Target)	7.7187
Number of days until at 90% of Target (using LCL)	11
Conclusion using Target Concentration:	**Stable for 11 days**
Average Day 0 Concentration	2352
LN(90% of Day 0 Concentration)	7.6576
Number of days until at 90% of Day 0 Concentration (using LCL)	22
Conclusion using Day 0 Concentration:	**Stable for 16 days**

[a] Significant at α = 0.05
[b] Not significant at α = 0.05

4.0 CONCLUSIONS

The chemical purity determined from Battelle-Sequim was 89.99% and from the manufacturer was 95.2%. Stability testing of methoxychlor in methylcellulose concluded that the chemical was stable at the average day 0 concentration of 2352 μg/mL for the required shipping and holding period of 2 weeks.

Chemical analyses of formulation samples and in-life samples were conducted for four target concentrations: 2.5, 5.0, 7.5, and 10.0 mg/mL (solutions were not prepared at Battelle-Sequim; the samples were returned from Research Triangle Institute). In-life chemical concentrations were determined for 24 samples collected during the 15 day period that the rats were dosed. Of the in-life samples that were analyzed for the target concentration of 5.0 mg/mL, the mean concentration measured was 5.26 mg/mL and the range of recovery was 73-161% of the target. Of the in-life samples that were analyzed for the target concentration of 7.5 mg/mL, the

mean concentration measured was 7.88 mg/mL and the range of recovery was 49-139% of the target. One of the twelve in-life samples from the 7.5 mg/mL target dose had poor internal standard recovery and therefore was not used in calculating the mean and range of recovery. CCV recoveries ranged from 92.3 to 102.8%. The MDL was 25 µg/mL.

APPENDIX A

MANUFACTURER'S CERTIFICATE OF ANALYSIS/PURITY

SIGMA

CF#1839

Certificate of Analysis

TEST	LOT {049H1328} RESULTS
Product Name	Methoxychlor
Product Number	M1501
CAS Number	72435
Formula	$C_{16}H_{15}Cl_3O_2$
Formula Weight	345.7
APPEARANCE	LIGHT ORANGE POWDER WITH A LIGHT TAN CAST
SOLUBILITY	CLEAR FAINT YELLOW SOLUTION AT 200 MG PLUS 4.0 ML OF ETHANOL
IR SPECTRUM	CONSISTENT WITH STRUCTURE
PURITY BY GAS CHROMATOGRAPHY	95.2%
QC ACCEPTANCE DATE	APRIL 1999

David Feldker, Manager
Analytical Services

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {062K0144} RESULTS
Product Name	Methylcellulose	
Product Number	M0512	
CAS Number	9004675	
APPEARANCE	WHITE WITH A YELLOW CAST POWDER	CONFORMS
SOLUBILITY	CLEAR TO HAZY COLORLESS TO LIGHT YELLOW VISCOUS SOLUTION AT 100 MG PLUS 5 ML OF WATER	VERY SLIGHTLY HAZY VERY FAINT YELLOW
VISCOSITY OF A 2% AQUEOUS SOLUTION	3500 TO 5600 CPS (20 DEG C)	4,202 CPS (SUPPLIER TEST RESULT)
QC ACCEPTANCE DATE		JULY 2002

David Feldker, Manager
Analytical Services

APPENDIX B

PURITY AND STABILITY TESTING PLAN

EDSP Purity Analysis and Stability Testing Plan for Methoxychlor

Chemical Name: Methoxychlor (MSL CF Login 1839)

CAS Number: 72-43-5

Lot Number: 49H1328
stored at RT in MSL5, Rm 219

Expiration date: 4/06

Manufacturer's Purity Information: 95.2%

Manufacturer's Stability Information: stable

MSL Purity Results:
Purity (%) To be determined at MSL by GC-FID scan

MDL has not been determined.

Bioassay Information:

Study Director
Name: Dr. Julia George
Affiliation: RTI
Location: RTP, NC
Telephone number: 919-541-5862

Proposed Bioassay: WA 2-28

Test Chemical: Methoxychlor

CAS: 72-43-5

Carrier(s): 0.25% methylcellulose (Sigma M0512) in DI water

Concentration: 2.5 mg/mL

Below MDL determined in Purity Analysis? Unknown.

In vitro or in *vivo tests*? *In vivo*

Organism to be tested: rat

Method of test solution administration: oral gavage

Planned/Proposed test duration: 6 weeks

Design of Stability Test: 2.5 mg/mL in glass at 4 deg. C in the dark for 42 days, analyzed weekly in triplicate by GC detector

Number of replicates: 3

Duration: 42 days, sampling each week

other factor:
Temperature regime(s): 4 deg. C
Test container type: glass
Light or dark: dark except when container is removed for sampling or handling
Other: none

Statistical testing: regression analysis of the slope for concentration versus time

Resulting records package:
Manufacturer's certificate of analysis or purity
MSDS
Records:

- date sample received;
- date(s) sample analyzed;
- sample matrix;
- electronic file identification codes (when applicable to identify instrument data files);
- data summary reports;
  - Chemical repository confirmatory test results of chemical identity and purity;
  - Chemical repository test results of lot-to-lot variation in chemical purity;
  - Chemical repository periodic assessment results of changes in purity of stock solutions and dilutions and generation of degradation products
- QC data reports;
- data qualifying flags; and
- dilution factor(s).

APPENDIX C

ANALYTICAL RESULTS OF STABILITY TESTING

Table C1. Methoxychlor concentration in Methylcellulose (µg/mL)

Target Conc.	Sample ID	Sampling Date	Methoxychlor (ug/mL)	Average	RSD	% Recovery
2500 µg/mL	1839-2a-4-1 Top	4/22/03	2328			
2500 µg/mL	1839-2a-4-1 Mid	4/22/03	2396	2352	1.64%	94.1%
2500 µg/mL	1839-2a-4-1 Bottom	4/22/03	2332			
Blank	Control 2	4/22/03	25 U			
2500 µg/mL	1839-2a-4-2 Top	4/24/03	2400			
2500 µg/mL	1839-2a-4-2 Mid	4/24/03	2331	2331	2.94%	93.3%
2500 µg/mL	1839-2a-4-2 Bottom	4/24/03	2263			
2500 µg/mL	1839-2a-4-3 Top	4/25/03	2286			
2500 µg/mL	1839-2a-4-3 Mid	4/25/03	2295	2290	0.28%	91.6%
2500 µg/mL	1839-2a-4-3 Bottom	4/25/03	Error in analysis			
Blank	Control 1	4/25/03	25 U			
Blank	Control 2	4/25/03	25 U			
Blank	Control 3	4/25/03	25 U			
2500 µg/mL	1839-2a-4-4 Top	4/28/03	2372			
2500 µg/mL	1839-2a-4-4 Mid	4/28/03	2356	2374	0.83%	95.0%
2500 µg/mL	1839-2a-4-4 Bottom	4/28/03	2395			
2500 µg/mL	1839-2a-4-5 Top	4/29/03	2387			
2500 µg /mL	1839-2a-4-5 Mid	4/29/03	2380	2359	1.77%	94.4%
2500 µg/mL	1839-2a-4-5 Bottom	4/29/03	2311			
2500 µg/mL	1839-2a-4-6 Top	4/30/03	2460			
2500 µg/mL	1839-2a-4-6 Mid	4/30/03	2465	2439	1.66%	97.6%
2500 µg/mL	1839-2a-4-6 Bottom	4/30/03	2392			
Blank	Control 4	4/30/03	25 U			
Blank	Control 5	4/30/03	25 U			
2500 µg mL	1839-2a-4-7 Top	5/1/03	2601			
2500 µg/mL	1839-2a-4-7 Mid	5/1/03	2548	2576	1.03%	103%
2500 µg/mL	1839-2a-4-7 Bottom	5/1/03	2580			
Blank	Control 6	5/1/03	25 U			
Blank	Control 7	5/1/03	25 U			
2500 µg/mL	1839-2a-4-8 Top	5/2/03	2172			
2500 µg/mL	1839-2a-4-8 Mid	5/2/03	2431	2330	5.94%	93.2%
2500 µg/mL	1839-2a-4-8 Bottom	5/2/03	2387			
Blank	Control 7	5/2/03	25 U			
Blank	Control 8	5/2/03	25 U			
2500 µg/mL	1839-2a-4-9 Top	5/6/03	2121			
2500 µg/mL	1839-2a-4-9 Mid	5/6/03	2228	2208	3.54%	88.3%
2500 µg/mL	1839-2a-4-9 Bottom	5/6/03	2274			
Blank	Control 9	5/6/03	25 U			
2500 µg/mL	1839-2a-4-10 Top	5/8/03	2275			
2500 µg/mL	1839-2a-4-10 Mid	5/8/03	2264	2294	1.86%	91.8%
2500 µg/mL	1839-2a-4-10 Bottom	5/8/03	2343			
Blank	Control 10	5/9/03	25 U			

Table C2. ICV/CCV Data for Methoxychlor Concentration in Methylcellulose

Date	Sample Name	Methoxychlor (µg/mL)	Recovery	PD
4/21/03	CCV 20 EDSP1	21.16	106%	5.80%
4/21/03	CCV 20 EDSP1	20.31	102%	1.55%
4/22/03	CCV 20 EDSP1	21.04	105%	5.20%
4/22/03	CCV 20 EDSP1	21.16	106%	5.80%
4/25/03	CCV 20 EDSP1	20.84	104%	4.20%
4/25/03	CCV 20 EDSP1	20.60	103%	3.00%
4/25/03	CCV 20 EDSP1	16.79	84.0%	16.1%
4/30/03	CCV 20 EDSP1	20.65	103%	3.25%
4/30/03	CCV 20 EDSP1	20.49	102%	2.45%
5/1/03	CCV 20 EDSP1	20.43	102%	2.15%
5/1/03	CCV 20 EDSP1	20.46	102%	2.30%
5/2/03	CCV 20 EDSP1	20.57	103%	2.85%
5/2/03	CCV 20 EDSP1	20.27	101%	1.35%
5/6/03	CCV 20 EDSP1	19.39	97.0%	3.05%
5/7/03	CCV 20 EDSP1	19.94	99.7%	0.30%
5/7/03	CCV 20 EDSP1	18.52	92.6%	7.40%
5/9/03	CCV 20 EDSP1	19.92	99.6%	0.40%
5/9/03	CCV 20 EDSP1	19.68	98.4%	1.60%
5/9/03	CCV 20 EDSP1	19.76	98.8%	1.20%

Text Box C1. Calibration Standard Preparation

Calibration Standard EDSP Mix 1
Calibrations were performed using a five-point calibration curve labeled EDSP Mix 1 A thru E. This mix is used for Atrazine, Fenarimol, p,p'-DDE, Methoxychlor and Vinclozolin analyzed by GC-FID. These standards were made by serial dilutions of standards for each compound. • Atrazine standard was made by weighing 0.0499 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with Methylene chloride and labeled 1826-1-1. • Fenarimol standard was made by weighing 0.0506 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with hexane and labeled 1829B-1. • p,p'-DDE standard was made by weighing 0.0501 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with hexane and labeled 1832-1a-1. • Methoxychlor standard was made by weighing 0.0513 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with hexane and labeled 1808-1-3. • Vinclozolin standard was made by weighing 0.0512 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with hexane and labeled 1779-78. This analysis used an internal standard, in this case 5a androstane, which is made by weighing 0.0511 g of the neat material into a 50 mL volumetric flask. This was then diluted to the 50 mL mark with hexane, this is then labeled REP7. The EDSP Mix 1 series (A through E) was made as follows. • Solution A, 1 ml of 1826-1-1, 1829B-1, 1832-1a-1, 1808-1-3, 1779-78 and 0.02 ml REP7 added to a 10 ml volumetric flask and diluted to the mark with hexane. • Solution B, 1 ml of 1826-1-1, 1829B-1, 1832-1a-1, 1808-1-3, 1779-78 and 1 ml REP7 added to a 50 ml volumetric flask and diluted to the mark with hexane. • Solution C, 0.25 ml of 1826-1-1, 1829B-1, 1832-1a-1, 1808-1-3, 1779-78 and 1 ml REP7 added to a 50 ml volumetric flask and diluted to the mark with hexane. • Solution D, 0.1 ml of 1826-1-1, 1829B-1, 1832-1a-1, 1808-1-3, 1779-78 and 2 ml REP7 added to a 100 ml volumetric flask and diluted to the mark with hexane. • Solution E, 0.05 ml of 1826-1-1, 1829B-1, 1832-1a-1, 1808-1-3, 1779-78 and 2 ml REP7 added to a 100 ml volumetric flask and diluted to the mark with hexane.

APPENDIX D

STATISTICAL REPORT

WA-2-28-03-01
Statistical Analysis conducted by Valerie Cullinan
Using Minitab Version 13.32, Minitab Inc., 1999.

———— 8/5/03 2:08:21 PM ————————————————————

Analysis of methoxychlor-2.5k in methylcellulose

- Test to determine if the sections are homogeneous.

Kruskal-Wallis Test: Methoxychlor versus section

```
Kruskal-Wallis Test on Methoxychlor

section     N    Median    Ave Rank         Z
1          10      2350        14.1     -0.41
2          10      2368        16.0      0.46
3           9      2343        14.9     -0.05
Overall    29                  15.0

H = 0.25  DF = 2  P = 0.882 NS – assume homogeneous and use sections as reps
```

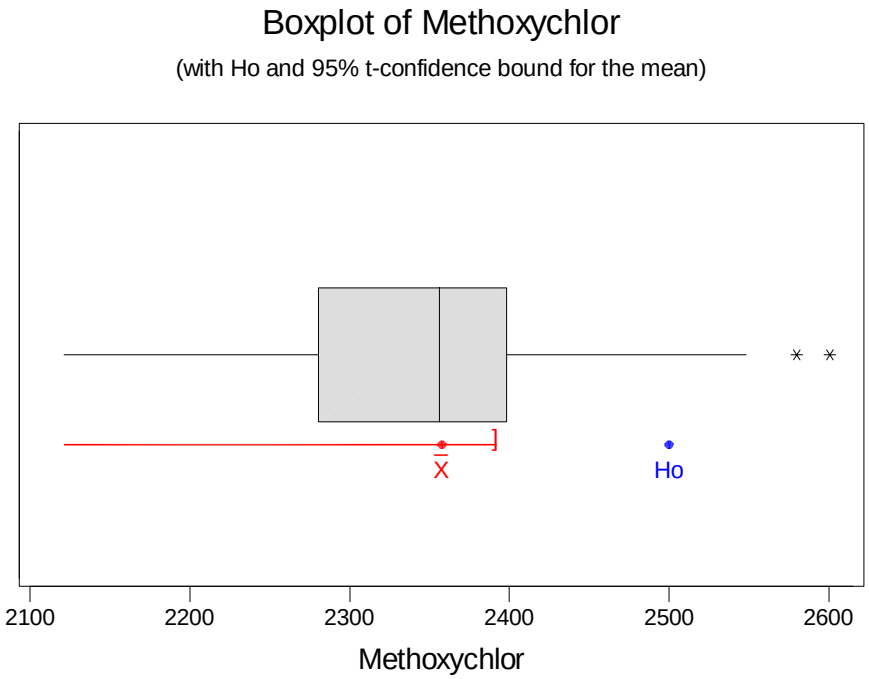
- Test to determine if the data are from a population with mean of 2500.

```
Macro performs a one-sample t-test for mu less than TARGET &
What is the target value for X    3
DATA> 2500
```

One-Sample T: Methoxychlor

```
Test of mu = 2500 vs mu < 2500

Variable          N      Mean     StDev   SE Mean
Methoxychlor     29    2357.7     109.0      20.2

Variable      95.0% Upper Bound        T      P
Methoxychlor              2392.1    -7.03  0.000
```

t Boxplot of Methoxychlor




```
Outliers are < Median-3*IQD OR > Median+3*IQD

Boundary for outliers are values <    2003.00  and >    2709.60

No outliers
```

- Transform data to natural logarithm and conduct regression analysis.

Day	Rep	Ln(Concentration)
0	1	7.7527
0	2	7.7817
0	3	7.7543
2	1	7.7832
2	2	7.7542
2	3	7.7243
3	1	7.7344
3	2	7.7385
6	1	7.7714
6	2	7.7648
6	3	7.7812
7	1	7.7777
7	2	7.7749
7	3	7.7456
8	1	7.8080
8	2	7.8098
8	3	7.7801
9	1	7.8635
9	2	7.8431
9	3	7.8555
10	1	7.6835
10	2	7.7961
10	3	7.7777

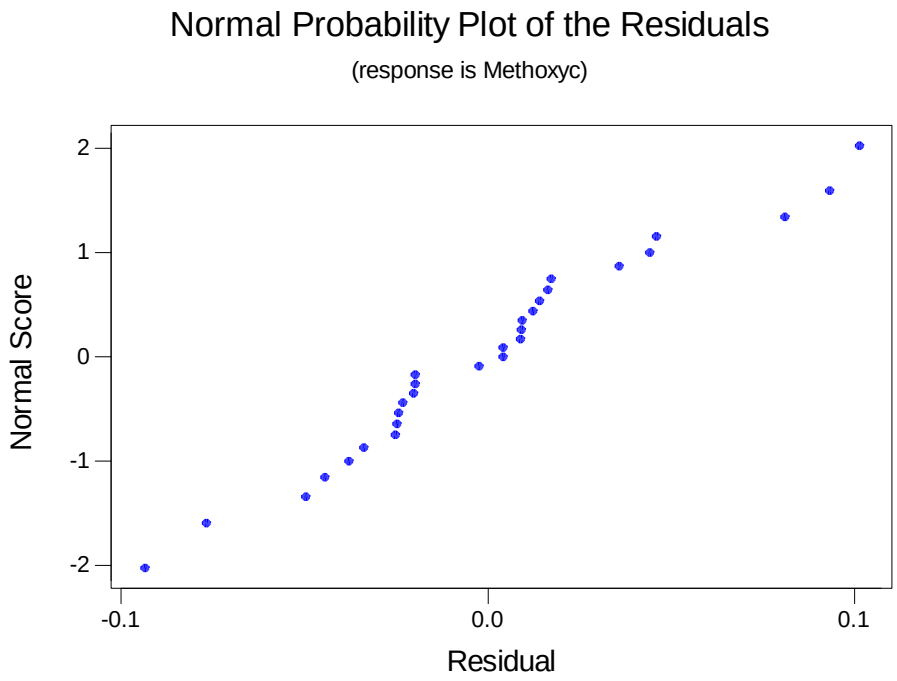
```
14       1      7.6598
14       2      7.7089
14       3      7.7292
16       1      7.7297
16       2      7.7248
16       3      7.7590
```

- Conducts Simple Linear Regression

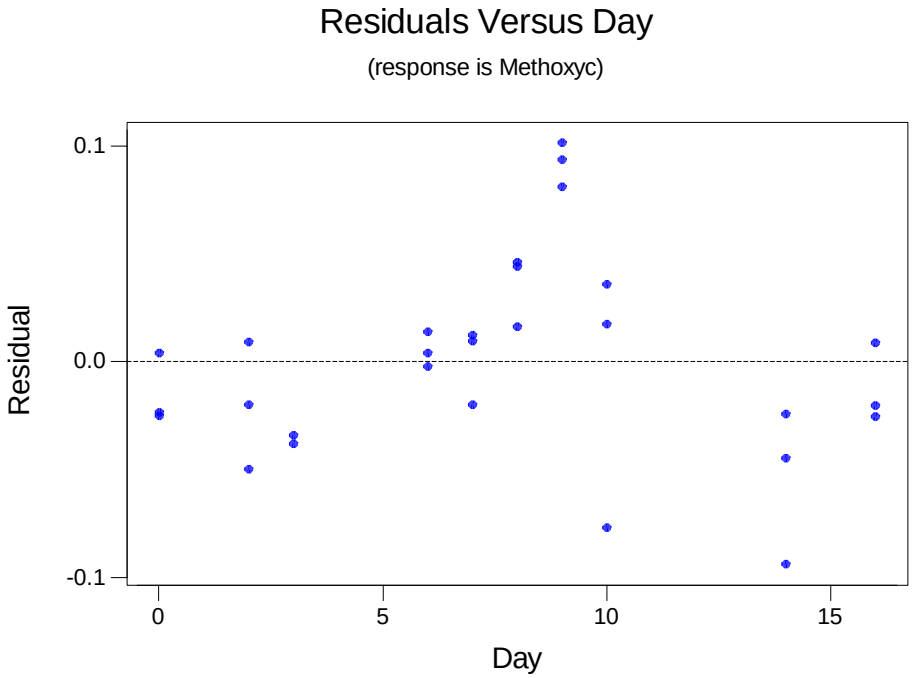
Regression Analysis: Methoxychlor versus Day

```
The regression equation is
Methoxychlor = 7.78 - 0.00172 Day

Predictor        Coef     SE Coef          T        P
Constant      7.77753     0.01606     484.43    0.000
Day         -0.001717    0.001774      -0.97    0.342       NS

S = 0.04614     R-Sq = 3.4%      R-Sq(adj) = 0.0%

Analysis of Variance

Source            DF          SS          MS         F        P
Regression         1    0.001994    0.001994      0.94    0.342
Residual Error    27    0.057471    0.002129
  Lack of Fit      8    0.043136    0.005392      7.15    0.000
  Pure Error      19    0.014335    0.000754
Total             28    0.059464

Unusual Observations
Obs        Day   Methoxychlor         Fit      SE Fit    Residual    St Resid
 18        9.0    7.86353     7.76208     0.00889     0.10144        2.24R
 20        9.0    7.85545     7.76208     0.00889     0.09337        2.06R
 24       14.0    7.65981     7.75350     0.01414    -0.09369       -2.13R

R denotes an observation with a large standardized residual
```

Normplot of Residuals for Methoxychlor



Residuals from Methoxychlor vs Day



- Power analysis for t-test of slope less than zero

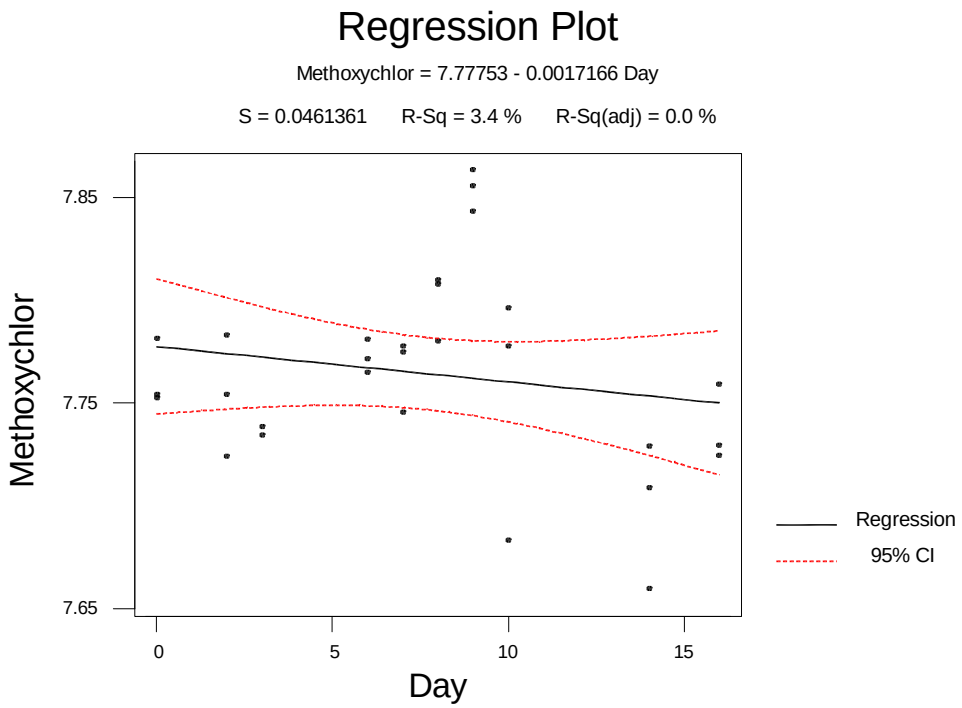
Power and Sample Size

```
1-Sample t Test

Testing mean = null (versus < null)
Calculating power for mean = null + difference
Alpha = 0.05  Sigma = 0.0461361

Sample
  Size   Power  Difference
    27  0.9900     -0.0362
```

- That means we would detect a mean of 7.788 as significantly less than ln(2500) = 7.824 or a change of 2411 from 2500 = 3.6% loss.

- Fit 95% confidence bands about the fitted simple linear model

Fitted Line Plot: Methoxychlor versus Day



- **Conclusion – stable for 16 days.**

APPENDIX E

CHEMISTRY RESULTS
FOR THE ANALYSIS OF IN-LIFE SAMPLES

QA/QC Narrative

PROJECT:	**EDSP WA 2-28**
PARAMETER:	Methoxychlor in a methylcellulose and water suspension in-life test solution samples
LABORATORY:	Battelle Marine Sciences Laboratory 1529 West Sequim Bay Rd. Sequim, WA 98382
MATRIX:	Methoxychlor in a methylcellulose and water suspension
TEST SOLUTION SAMPLE CUSTODY AND PROCESSING:	Battelle, Sequim did not prepare the test solutions used in the WA 2-28 study. However, 45 g of the test chemical methoxychlor (CF 1839-2a, lot #49H1328, exp. 4/06) was sieved through an 80-mesh screen and shipped to RTI. Test solutions were prepared by RTI. Samples returned for analysis included two sets of samples to assess homogeneity of the test solutions prepared at RTI (top, middle, bottom) and two samples to assess dosage preparation at 5.0 and 7.5 mg/mL concentrations. In addition, two sample sets to verify the range of test sample concentrations (2.5, 5.0, 7.5, and 10.0 mg/mL) prepared on 5/27/03 and 5/30/03 were submitted for analysis. In-life samples at first and last dosing for test solution formulations prepared on 5/17/03, 5/23/03, and 5/30/03 were also returned for analysis. There were no control samples (0.00 mg/mL concentration) returned for analysis. Samples returned in scintillation vials were processed as follows: The vial was weighed and the content pipetted into a 25 mL amber glass container. First, 10 mL methylene chloride (JT Baker Lot # 36E04) was added to the scintillation vial, agitated, then added to the amber glass container. Then 15 mL methylene chloride (JT Baker Lot # 36E04) was added to the scintillation vial, agitated, and then added to the amber container. A total of 25 mL methylene chloride was used. The rinsed vials were allowed to dry, and the weight of the empty scintillation vials was determined so that the sample weight could be calculated from the difference of the two weights measured. 2.0 g NaCl (EM Science lot # 303OB06) was added to the sample and this was shaken by hand for 2 minutes. 0.2 mL was removed from the amber glass container and pipetted into a 1.8 mL auto sampler vial with 0.02 mL internal standard, PP-1219 (1004 ug/mL, 5a androstane) and 0.78 mL of hexane (JT Baker, lot # 40E12).

	The Chain of Custody documentation received from RTI did not list samples individually and sample IDs are not unique. The target concentrations for the in-life samples and the identification of replicates submitted by RTI were not documented in the chain of custody information accompanying the samples.
TEST SOLUTION SAMPLE ANALYSIS	Samples were analyzed using a gas chromatograph with a flame ionization detector (GC-FID) using a Phenomenex Zebron ZB-5 column (30 m x 0.25 mm) with a 0.25 um coating. The auto sampler was set to inject a 1 uL sample. The injection port temperature was set at 270^{o}C. The oven temperature was set at 320^{o}C, with splitless injection and 50^{o}C initial temperature, which was held for 1.5 min and ramped at 20^{o}C/min to the 320^{o}C limit, which was held for 10 minutes. Calibration was done using dilutions of analytical standard EDSP Mix 1. GC-FID data are stored on the computer, WD35603, in room 223, MSL 5.

Data Quality Objectives:	Control Limits
Procedural Blank	< 5 * MDL
Continuing Standard Recovery	75-125%

IN-LIFE QC DATA SUMMARY

METHODS:	GC-FID using a Phenomenex Zebron ZB-5 column (30 m x 0.25 mm) with a 0.25 um coating.
CALIBRATION:	Calibration was done using a 5-point calibration curve from an EDSP Mix 1 preparation.
CONTINUING STANDARD RECOVERY:	Eight CCVs were analyzed with in-life samples. CCV recoveries ranged from 92 to 103% with a mean of 97.6%. All recoveries were within the acceptance criteria of $\pm$ 25%.
BLANK	Six blanks were analyzed with in-life samples. Of these, 5 blanks were non-detects with no peaks measured (reported as zero) and one had a quantifiable amount of methoxychlor (5.62 ug/mL), which was well below the MDL and reported as 25U.
BLANK SPIKES	Three blanks were spiked at 2.03 mg/mL. Recoveries were 93.1, 96.8 and 87.1 %, all within the generally adopted acceptable limits of 40-120% for organic sample blank spikes.
INTERNAL STANDARD	Seventeen internal standard recoveries of 5a androstane were evaluated. One was of concern (5.32%) for sample 2026-26b suggesting that the data should not be used. The associated sample value also seemed lower than expected, resulting in the recommendation to qualify the data value for sample 2026-26b as a "Q" questionable value. Based on the remaining 16 IS recoveries, the recoveries ranged from 93 to 100% with a mean of 96.1% recovery, well within the generally adopted 40-120% acceptance criteria for organic sample internal surrogates.
DETECTION LIMIT:	The method detection limit for methoxychlor for in-life sample analysis is25 ug/mL.
HOLDING TIME:	The statistical analysis assessing the stability of methoxychlor in a methylcellulose suspension for WA 2-28 is pertinent. The analysis of the data demonstrated that the test solution was homogeneous. The stability test was conducted over a 16-day period because the protocol in use by RTI specifies that test solutions are stable for 5 days, so there was no question that the test solution would be made every 5 days as per the protocol. Therefore, the two week period for stability testing was determined and selected based on an estimate of the time needed to ship and analyze in-life samples, about 2 weeks. Basically, then the stability study became a holding time evaluation. The target concentration of 2500 µg/mL was evaluated against the 16-day average, 2358 µg/mL, and the data were assessed to determine if the test concentration was

	maintained at 90% of the target. The conclusion was that test solution was stable only for 11 days. A subsequent analysis using the average Day 0 concentration (2352 µg/mL) as the basis for evaluating if the test concentration was maintained at 90% of the target, extended the stability to 16 days. The average concentration based on all the data (2358 µg/mL, n=29) and the Day 0 average (2352 µg/mL, n=3) are not very different, so this approach seems feasible. A predictive regression model using natural log transformed data was used to determine the stability. The times of sample collection, receipt, and analysis for the in-life data are provided in Table E1. All samples analyzed within 11 days of receipt. Some samples were analyzed after the documented stability time of two weeks.

Table E1. In-life data from samples prepared by and provided to Battelle, Sequim by RTI.

MSL Sample ID	RTI Sample ID	Sample Description	Target Concentration (mg/mL)	Methoxychlor (mg/mL)	Percent Recovery Based on Target	Prep Date	Collection Date	Received At MSL	Analyzed
2026-4	10303-90-16	dosage analysis	5.0	4.37	87%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-5	10303-90-17	dosage analysis	7.5	6.75	90%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-1	10303-90-13	homogeneity-top	2.5	2.21	89%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-2	10303-90-14	homogeneity-middle	2.5	2.45	98%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-3	10303-90-15	homogeneity-bottom	2.5	2.34	94%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
			Mean	2.33	94%				
			CV	4.81%					
2026-6	10303-90-18	homogeneity-top	10	9.32	93%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-7	10303-90-19	homogeneity-middle	10	10.3	103%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
2026-8	10303-90-20	homogeneity-bottom	10	10.4	104%	5/17/2003	5/17/2003	5/20/2003	5/21/2003
			Mean	10.0	100%				
			CV	5.97%					
2026-9	10303-92-09	dosage analysis	2.5	2.48	99%	5/23/2003	5/23/2003	5/28/2003	6/6/2003
2026-10	10303-92-10	dosage analysis	5.0	5.63	113%	5/23/2003	5/23/2003	5/28/2003	6/6/2003
2026-11	10303-92-11	dosage analysis	7.5	8.15	109%	5/23/2003	5/23/2003	5/28/2003	6/6/2003
2026-12	10303-92-12	dosage analysis	10.0	10.1	101%	5/23/2003	5/23/2003	5/28/2003	6/6/2003
2026-13	10303-94-09	dosage analysis	2.5	2.63	105%	5/30/2003	5/30/2003	6/3/2003	6/6/2003
2026-14	10303-94-10	dosage analysis	5.0	5.23	105%	5/30/2003	5/30/2003	6/3/2003	6/6/2003
2026-15	10303-94-11	dosage analysis	7.5	7.85	105%	5/30/2003	5/30/2003	6/3/2003	6/6/2003
2026-16	10303-94-12	dosage analysis	10.0	10.3	103%	5/30/2003	5/30/2003	6/3/2003	6/6/2003
2026-17a	10303-90-02	First day of dosing sample	5.0	5.14	103%	5/17/2003	5/20/2003	6/10/2003	6/12/2003
2026-17b	10303-90-02	First day of dosing sample	5.0	5.23	105%	5/17/2003	5/20/2003	6/10/2003	6/12/2003
2026-18a	10303-90-03	First day of dosing sample	7.5	7.88	105%	5/17/2003	5/20/2003	6/10/2003	6/12/2003

MSL Sample ID	RTI Sample ID	Sample Description	Target Concentration (mg/mL)	Methoxychlor (mg/mL)	Percent Recovery Based on Target	Prep Date	Collection Date	Received At MSL	Analyzed
2026-18b	10303-90-03	First day of dosing sample	7.5	7.79	104%	5/17/2003	5/20/2003	6/10/2003	6/12/2003
2026-19a	10303-90-02	Lat day of dosing sample	5.0	6.14	123%	5/17/2003	5/25/2003	6/10/2003	6/12/2003
2026-19b	10303-90-02	Lat day of dosing sample	5.0	8.07	161%	5/17/2003	5/25/2003	6/10/2003	6/12/2003
2026-20a	10303-90-03	Lat day of dosing sample	7.5	10.4	139%	5/17/2003	5/25/2003	6/10/2003	6/13/2003
2026-20b	10303-90-03	Lat day of dosing sample	7.5	10.1	135%	5/17/2003	5/25/2003	6/10/2003	6/13/2003
2026-21a	10303-92-02	First day of dosing sample	5.0	5.01	100%	5/23/2003	5/26/2003	6/10/2003	6/13/2003
2026-21b	10303-92-02	First day of dosing sample	5.0	5.31	106%	5/23/2003	5/26/2003	6/10/2003	6/13/2003
2026-22a	10303-92-03	First day of dosing sample	7.5	7.79	104%	5/23/2003	5/26/2003	6/10/2003	6/13/2003
2026-22b	10303-92-03	First day of dosing sample	7.5	7.39	99%	5/23/2003	5/26/2003	6/10/2003	6/13/2003
2026-23a	10303-92-02	Lat day of dosing sample	5.0	3.67	73%	5/23/2003	6/1/2003	6/10/2003	6/13/2003
2026-23b	10303-92-02	Lat day of dosing sample	5.0	3.75	74%	5/23/2003	6/1/2003	6/10/2003	6/13/2003
2026-24a	10303-92-03	Lat day of dosing sample	7.5	8.08	108%	5/23/2003	6/1/2003	6/10/2003	6/13/2003
2026-24b	10303-92-03	Lat day of dosing sample	7.5	7.63	102%	5/23/2003	6/1/2003	6/10/2003	6/13/2003
2026-25a	10303-94-02	First day of dosing sample	5.0	4.46	89%	5/30/2003	6/2/2003	6/10/2003	6/13/2003
2026-25b	10303-94-02	First day of dosing sample	5.0	4.16	83%	5/30/2003	6/2/2003	6/10/2003	6/13/2003
2026-26a	10303-94-03	First day of dosing sample	7.5	3.67	49%	5/30/2003	6/2/2003	6/10/2003	6/13/2003
2026-26b	10303-94-03	First day of dosing sample	7.5	2.83 Q	38%	5/30/2003	6/2/2003	6/10/2003	6/13/2003
2026-27a	10303-94-02	Lat day of dosing sample	5.0	6.10	122%	5/30/2003	6/5/2003	6/10/2003	6/16/2003
2026-27b	10303-94-02	Lat day of dosing sample	5.0	6.13	123%	5/30/2003	6/5/2003	6/10/2003	6/16/2003
2026-28a	10303-94-03	Lat day of dosing sample	7.5	7.72	103%	5/30/2003	6/5/2003	6/10/2003	6/16/2003
2026-28b	10303-94-03	Lat day of dosing sample	7.5	8.26	110%	5/30/2003	6/5/2003	6/10/2003	6/16/2003

Calculations were conducted before rounding

Q Questionable value due to poor recovery of the associated internal standard; data are not included in summary statistics

Appendix III

Feed Analysis Reports

Certified Rodent Diet 5002*

DESCRIPTION

Certified Rodent Diet is a Constant Nutrition™ formulation that has yielded highly favorable results for the maintenance, growth and reproduction of rats and mice. It has been developed as a complete life-cycle diet that can also be used by breeders to assure animals do not develop undesirable tissue residues of contaminants. A sample of this product will have been assayed prior to shipment.

Features and Benefits

- Each package is assayed for environmental contaminants prior to shipment
- Preanalysis monitoring assures maximum diet control
- Fulfills GLP requirements

Product Forms Available

- Oval pellet, 10 mm x 16 mm x 25 mm length (3/8"x5/8"x1")
- Meal (ground pellets)

GUARANTEED ANALYSIS

Crude protein not less than	20.0%
Crude fat not less than	4.5%
Crude fiber not more than	5.5%
Ash not more than	7.0%
Added minerals not more than	2.5%

INGREDIENTS

Ground corn, dehulled soybean meal, ground wheat, fish meal, wheat middlings, brewers dried yeast, cane molasses, wheat germ, dried beet pulp, dehydrated alfalfa meal, ground oats, dried whey, ground soybean hulls, soybean oil, calcium carbonate, casein, salt, dicalcium phosphate, choline chloride, DL-methionine, cholecalciferol, vitamin A acetate, pyridoxine hydrochloride, dl-alpha tocopheryl acetate, thiamin mononitrate, nicotinic acid, calcium pantothenate, riboflavin, cyanocobalamin, folic acid, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate.

FEEDING DIRECTIONS

Feed ad libitum to rodents. Plenty of fresh, clean water should be available to the animals at all times. Refer to the "Animal Care and Biological Values" section of this manual for detailed feeding directions.

Rats- All rats will eat varying amounts of feed depending on their genetic origin. Larger strains will eat between 15-30 grams per day. Smaller strains will eat between 12-15 grams per day. Feeders in rat cages should be designed to hold two to three days supply of feed at one time.

Mice-Adult mice will eat 4 to 5 grams of pelleted ration daily. Some of the larger strains may eat as much as 8 grams per day per animal. Feed should be available on a free choice basis in wire feeders above the floor of the cage.

Hamsters-Adults will eat 10 to 14 grams per day.

CHEMICAL COMPOSITION[1]

Nutrients[2]

Protein, %	**20.1**
Arginine, %	1.13
Cystine, %	0.27
Glycine, %	0.86
Histidine, %	0.49
Isoleucine, %	1.03
Leucine, %	1.58
Lysine, %	1.18
Methionine, %	0.43
Phenylalanine, %	0.88
Tyrosine, %	0.59
Threonine, %	0.78
Tryptophan, %	0.24
Valine, %	1.05
Serine, %	1.01
Aspartic Acid, %	2.19
Glutamic Acid, %	4.20
Alanine, %	1.24
Proline, %	1.47
Taurine, %	0.01
Fat (ether extract), %	**4.5**
Fat (acid hydrolysis), %	**5.1**
Cholesterol, ppm	150
Linoleic Acid, %	2.15
Linolenic Acid, %	0.16
Arachidonic Acid, %	<0.01
Omega-3 Fatty Acids, %	0.34
Total Saturated Fatty Acids, %	0.86
Total Monounsaturated Fatty Acids, %	1.14
Fiber (Crude), %	**4.6**
Neutral Detergent Fiber[3], %	13.8
Acid Detergent Fiber[4], %	5.9
Nitrogen-Free Extract (by difference), %	**55.0**
Starch, %	36.3
Glucose, %	0.25
Fructose, %	0.30
Sucrose, %	3.13
Lactose, %	1.11
Total Digestible Nutrients, %	**77.0**
Gross Energy, kcal/gm	**4.04**
Physiological Fuel Value[5], kcal/gm	**3.41**
Metabolizable Energy, kcal/gm	**3.10**

Minerals

Ash, %	**5.8**
Calcium, %	0.80
Phosphorus, %	0.60
Phosphorus (non-phytate), %	0.34
Potassium, %	0.86
Magnesium, %	0.21
Sulfur, %	0.25
Sodium, %	0.30
Chlorine, %	0.47
Fluorine, ppm	13
Iron, ppm	210
Zinc, ppm	76
Manganese, ppm	75
Copper, ppm	11
Cobalt, ppm	0.6
Iodine, ppm	0.77
Chromium, ppm	2.0
Selenium, ppm	0.25

Vitamins

Carotene, ppm	5.6
Vitamin K (as menadione),ppm	0.4
Thiamin Hydrochloride, ppm	16
Riboflavin, ppm	8.0
Niacin, ppm	95
Pantothenic Acid, ppm	17
Choline Chloride, ppm	1800
Folic Acid, ppm	4.0
Pyridoxine, ppm	6.0
Biotin, ppm	0.13
B_{12}, mcg/kg	20
Vitamin A, IU/gm	18
Vitamin D_3 (added), IU/gm	2.2
Vitamin E, IU/kg	66

Calories provided by:

Protein, %	23.585
Fat (ether extract), %	11.880
Carbohydrates, %	64.535

*Product Code

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.
2. Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.
3. NDF = approximately cellulose, hemi-cellulose and lignin.
4. ADF = approximately cellulose and lignin.
5. Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbo- hydrate (use Nitrogen Free Extract) x 4,9,4 kcal/gm respectively.

PRODUCT: CERT. RODENT DIET
Code: 5002 Lot Number: FEB 10 03 2A - A

IFSP

Isoflavone profile, saponification

Daidzin	170	ppm
Daidzein	6.0	ppm
Total Daidzein Compounds	176	ppm
Genistin	182	ppm
Genistein	10.0	ppm
Total Genistein Compounds	192	ppm
Glycitin	52.0	ppm
Glycitein	12.0	ppm
Total Glycitein Compounds	64.0	ppm
Total Isoflavones	432	ppm
Daidzin (Aglycone Units)	104	ppm
Daidzein (Aglycone Units)	6.0	ppm
Total Daidzein (Aglycone Units)	110	ppm
Genistin (Aglycone Units)	114	ppm
Genistein (Aglycone Units)	10.0	ppm
Total Genistein (Aglycone Units)	124	ppm
Glycitin (Aglycone Units)	33.0	ppm
Glycitein (Aglycone Units)	12.0	ppm
Total Glycitein (Aglycone Units)	45.0	ppm
Total Isoflavones (Aglycone Units)	279	ppm

NOTE: Results based on analysis using 5 gram sample

Controls runs with these samples:

Ctrl 1 = 988 ppm; limits: 907-1028 ppm

Ctrl 2 = 1849 ppm; limits: 1690-1890 ppm

PRODUCT: CERT. RODENT DIET
Code: 5002 Lot Number: FEB 10 03 2A - B

IFSP

Isoflavone profile, saponification

Daidzin	177	ppm
Daidzein	6.0	ppm
Total Daidzein Compounds	183	ppm
Genistin	184	ppm
Genistein	9.0	ppm
Total Genistein Compounds	193	ppm
Glycitin	53.0	ppm
Glycitein	2.0	ppm
Total Glycitein Compounds	55.0	ppm
Total Isoflavones	431	ppm
Daidzin (Aglycone Units)	108	ppm
Daidzein (Aglycone Units)	6.0	ppm
Total Daidzein (Aglycone Units)	114	ppm
Genistin (Aglycone Units)	115	ppm
Genistein (Aglycone Units)	9.0	ppm
Total Genistein (Aglycone Units)	124	ppm
Glycitin (Aglycone Units)	34.0	ppm
Glycitein (Aglycone Units)	2.0	ppm
Total Glycitein (Aglycone Units)	36.0	ppm
Total Isoflavones (Aglycone Units)	274	ppm

NOTE: Results based on analysis using 5 gram sample

Controls runs with these samples:

Ctrl 1 = 988 ppm; limits: 907-1028 ppm

Ctrl 2 = 1849 ppm; limits: 1690-1890 ppm

PRODUCT: CERT. RODENT DIET
Code: 5002 Lot Number: FEB 10 03 2A - C

IFSP

Isoflavone profile, saponification

Daidzin	175	ppm
Daidzein	6.0	ppm
Total Daidzein Compounds	181	ppm
Genistin	180	ppm
Genistein	10.0	ppm
Total Genistein Compounds	190	ppm
Glycitin	53.0	ppm
Glycitein	10.0	ppm
Total Glycitein Compounds	63.0	ppm
Total Isoflavones	434	ppm
Daidzin (Aglycone Units)	107	ppm
Daidzein (Aglycone Units)	6.0	ppm
Total Daidzein (Aglycone Units)	113	ppm
Genistin (Aglycone Units)	113	ppm
Genistein (Aglycone Units)	10.0	ppm
Total Genistein (Aglycone Units)	123	ppm
Glycitin (Aglycone Units)	34.0	ppm
Glycitein (Aglycone Units)	10.0	ppm
Total Glycitein (Aglycone Units)	44.0	ppm
Total Isoflavones (Aglycone Units)	280	ppm

NOTE: Results based on analysis using 5 gram sample

Controls runs with these samples:

Ctrl 1 = 988 ppm; limits: 907-1028 ppm

Ctrl 2 = 1849 ppm; limits: 1690-1890 ppm

Reviewed by CSS ; Levels are acceptable 10-6-03

Appendix IV

Protocol and Amendment

EPA Contract No.: 68-W-01-023 (Battelle Prime Contractor)
EPA Work Assignment No.: WA-28
RTI Contract No.: 65U-08055.001.021
RTI Study Code: Rt03-ED07
RTI Master Protocol No.: RTI-871

TITLE: 15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley (CD®) Rats

SPONSOR: Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

TESTING FACILITY: RTI International
Chemistry and Life Sciences
Center for Life Sciences and Toxicology
Post Office Box 12194
Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: March 2003
PROPOSED EXPERIMENTAL TERMINATION DATE: July 2003

AMENDMENTS:

Number	Date	Section(s)	Page(s)
1			
2			
3			
4			
5			

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Please note: Jim Kariya was erroneously listed as the EPA WAM. A pen-in-ink correction was made to show Gary Timm as the WAM and Gary Timm signed as the EPA WAM on 2/19/2003.
This notation added 2/20/2003 by Gary E Timm

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Attachment: Material Safety Data Sheets (MSDSs)

Linuron
Methoxychlor

1.0 OBJECTIVE AND BACKGROUND

The objectives of this study are to

1) evaluate the design of an *in vivo* screen for identifying the mode of action of potential endocrine modulators in adult male rats, using a collection of endpoints;

2) characterize the response of this endocrine screen using two compounds with known endocrine activity.

The Food Quality Protection Act of 1996 requires the EPA to develop and implement a screening program using valid tests for determining the potential in humans for estrogenic effects from pesticides. This program has been expanded on the advice of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to include androgenic (and anti-androgenic) effects and effects from thyroid-hormone (TH)-like (and anti-TH) substances (EDSTAC, 1998). EPA proposed a two-tiered screening program in a Federal Register notice in 1998 (63 FR 71542-71568, Dec. 28, 1998) that covered not only pesticides but also commercial chemicals subject to regulation under the Toxic Substances Control Act (TSCA; 15 USC 2601) and environmental and drinking water contaminants. One of the assays recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as an alternate assay was a short term screen in an intact adult male. The adult male assay was developed to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; 5α-reductase inhibitors; steroid biosynthesis inhibitors; or compounds that alter thyroid function. Results from this assay and/or with the use of ip injection as the route of administration, and other assays with a similar purpose, have been reported (O'Connor et al., 1996, 1999, 2002a,b).

Based on the EDSTAC's recommendations, one of the assays that the EPA has proposed to validate as a potential alternative for other assays in the Tier 1 battery in an endocrine disruptor screening program is a male *in vivo* assay (see FR Vol. 63, No. 248, pp. 71541-71568, December 28, 1998). The utility of this battery for screening unknown compounds for endocrine activity will be evaluated.

2.0 MATERIALS AND METHODS

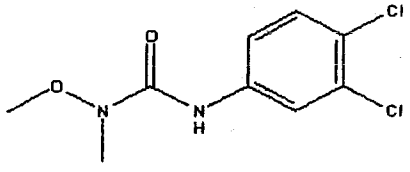
2.1 TEST SUBSTANCES

2.1.1 Linuron

CAS Number 330-55-2

Synonyms: Linuron ; N-(3,4-Dichlorophenyl)-N'-methoxy-N'-methylurea; Linuron; Lorox; Afalon; Linurex; N'-(3,4-Dichlorophenyl)-N-methoxy-N-methylurea; methoxydiuron; du Pont Herbicide 326; Hoe 2810; Linorox; Sarclex; Aflon; Linex 4L; Lorox 4L; Lorox 50W; Lorox DF; Lorox L; Lorox Plus; Alafon; Linex; Lorax; 1-Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; Urea, 1-(3,4-dichlorophenyl)-3-methoxy-3-methyl-; Garnitan; Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; Premalin; Cephalon; 3-(3,4-Dichlorophenyl)-1-methoxy(methyl)urea;

Structure:



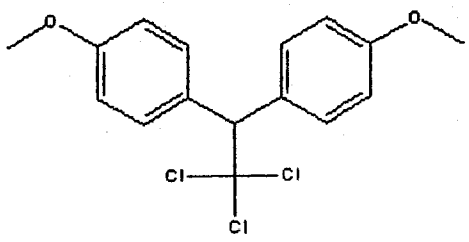
Supplier:	Chem Services
Lot Number:	273-81B
Purity:	99%
Appearance:	Crystalline solid
Molecular Formula:	$C_9H_{10}Cl_2N_2O_2$
Molecular Weight:	249
Storage, Bulk Chemical:	Room Temperature
Storage, Test Solution:	4 deg C

2.1.2 Methoxychlor

CAS Number 72-43-5

Synonyms: 1,1'-(2,2,2-Trichloroethylidene)-bis[4-methoxybenzene]; 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane; Methoxychlor; 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane; Marlate; Metox; Chemform; DMDT; Methoxy DDT; Maxie; Maralate; 2,2-bis(p-anisyl)-1,1,1-trichloroethane; 1,1-bis(p-methoxyphenyl)-2,2,2-trichloroethane; dianisyltrichloroethane; p,p'-dimethoxydiphenyltrichloroethane; di(p-methoxyphenyl) trichloromethyl methane; 2,2,2-trichloro-1,1-bis(4-methoxyphenyl)ethane; dimethoxy-ddt; methoxcide; methoxo; dimethoxy-dt; p,p'-dmdt; flo pro mcseed protectant; p,p'-methoxychlor; Moxie; oms 466; 4,4-(2,2,2-trichloroethylidene)dianisole; double-m ec; methoxychlor 2 ec; mezox k; 1,1,1-Trichloro-2,2-bis(p-anisyl)ethane; Bis(p-anisyl)-1,1,1-trichloroethane; Bis(p-methoxyphenyl)-1,1,1-trichloroethane; Dimethoxydiphenyltrichloroethane; Chemform methoxychlor;

Structure:



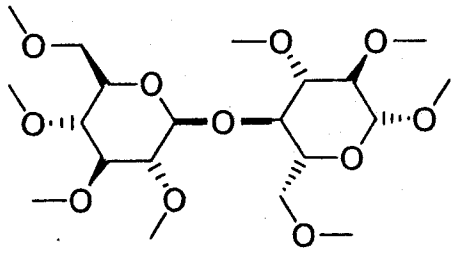
Supplier:	Sigma
Lot Number:	49H1328
Purity:	95.2%
Appearance:	Light orange powder
Molecular Formula:	$C_{16}H_{15}Cl_3O_2$
Molecular Weight:	345.7
Storage, Bulk Chemical:	Room Temperature
Storage, Test Solution:	4 deg C

2.1.3 Vehicle: Methylcellulose

CAS number: 9004-67-5

Synonyms: Cologel; Celevac; tylose mh20; tylose mh50; tylose mh300; tylose mh1000; tylose mh2000; tylose mh4000; tylose mh300p; tylose sap; tylose sl; tylose sl 100; tylose sl 400; tylose sl 600; tylose twa; methylcellulose; viscol; viscontran 152; viscosol; walsroder mc 20000s; methyl ether cellulose; adulsin; bagolax; bufapto methalose; bulkaloid; celacol m; celacol m20; celacol m450; celacol mm; celacol mm 10p; celacol m 20p; cellapret; cellogran; cellothyl; cellulose methyl; cellulose methylate; cellumeth; cethylose; cethytin; culminal k 42; edisol m; hydrolose; mapolose m25; mapolose 60sh50; mco 8000; mc 4000 cp; mc 20000s; mellose; methocel 10; methocel 15; methocel 181; methocel 400; methocel 4000; methocel a; methocel chg; methocel 400cps; methocel 4000cps; methocel mc; methocel mc 25; methocel mc4000; methocel mc 8000; methocel sm 100; methulose; methyl cellulose-a; methyl cellulose ether; metolose mc 8000; metolose 60sh; metolose 60sh400; metolose sm 15; metolose sm 100; metolose sm 4000; mmts-btr; napolone; Nicel; rhomellose; syncelose; tylose 444; tylose A4S; tylose mf; tylose mh; Cellulose, methyl ether; Citrucel; Methyl cellulose (viscosity: ca 15 cP (2% solution in water)); Methylcel MC;

Structure:



Supplier:	(to be added by sponsor)
Lot Number:	(to be added by sponsor)
Purity:	(to be added by sponsor)
Appearance:	(to be added by sponsor)
Molecular Formula:	$(C_7H_{14}O_5)_x$ (polymer)
Molecular Weight:	40,000 to 180,000 (polymer)
Storage, Bulk Chemical:	(to be added by sponsor)
Storage, Vehicle Solution:	(to be added by sponsor)

Note: **Chemical information not currently available will be added by amendment.**

2.2 CHEMICAL SAFETY AND HANDLING

See MSDSs of all chemicals in Attachment.

2.3 DOSE FORMULATION AND ANALYSIS

The dosing formulations will be prepared at a frequency determined by stability tests initiated prior to the start of the study. Formulations will be prepared at the Battelle Chemical Repository, Sequim, WA, and stored in wide-mouth, 200 ml amber bottles. They will be shipped on ice or with frozen cold packs via 24-hour express delivery and logged into the RTI Materials Handling Facility. Prior to transfer to the Reproductive and Developmental Toxicology Laboratory for dosing, the concentration of the dose formulations will be verified by the Battelle Chemical Respository. The test materials will be mixed in 0.25% aqueous methylcellulose, with the concentration determined by the following formula:

$$\text{Concentration (mg / ml)} = \frac{\text{Dose per time (mg / kg)}}{\text{Dosage volume per time (5 ml / kg)}}$$

An aliquot of each dose level per formulation will be analyzed by Battelle. The dosing bottles will be identified at RTI by a five-digit random number Rx code, and a color code. Personnel, other than the Laboratory Supervisor, Project Toxicologist, and Study Director, will not be informed of the test chemicals or formulation concentrations until all laboratory work is completed (i.e., the study technicians will be "blind" for chemical and dose). The dosing formulations will be stored at refrigerated temperatures. Prior to dosing each day, the formulations will be removed from the refrigerator. Solutions will be shaken and warmed to room temperature. Aliquots (at least 5 ml) from the dosing bottles will be collected on the first day of dosing (SD 0), the eighth day of dosing (SD 7) and the last day of dosing (SD 14). These samples will be held in refrigerated storage until the end of the dosing period, when they will be shipped overnight on frozen cold packs to Battelle Chemical Repository, Sequim, WA, for analysis.

2.4 ANIMALS

2.4.1 Species and Supplier

The proposed test animals will be the Sprague Dawley Derived Outbred Albino Rat Crl:CD®(SD) IGS BR supplied by Charles River Laboratories, Inc., Raleigh, NC.

2.4.2 Live Animals and Species Justification

The use of live animals has been requested by the Sponsor. Alternative test systems are not available for the assessment of effects of chemicals on reproduction and development in intact mammals for determining the potential risk for humans from endocrine-mediated effects of pesticides and other chemicals. The Charles River CD® rat has been the subject of choice on reproductive and developmental toxicology contracts at RTI since 1976, and has been used for other toxicology studies with these test materials. Large historical data bases for growth, food, and water consumption are available from the supplier. The Crl:CD®(SD)IGS BR rat has been selected on the basis of extensive experience with this strain and its suitability with respect to sensitivity to endocrine modulators. This study does not unnecessarily duplicate any previous study.

2.4.3 Total Number, Age, and Weight

Adult male Crl:CD®(SD)IGS BR rats, approximately 10 weeks of age and weighing between 326 and 350 grams, will be purchased specifically for this study. A total of 150 rats will be procured for this study.

2.4.4 Quality Control

The shipment of males will be quarantined on arrival, and quality control evaluation will be initiated within one day after receipt. Within one day after receipt, two male rats will be chosen from the shipment, sacrificed, and blood collected for assessment of viral antibody status.

Heat-inactivated serum will be sent to BioReliance (Rockville, MD) for their Level 1 Rat Antibody Screen. The viral screen will consist of evaluation for the presence of antibodies against the following: Toolan H-1 virus (H-1), Sendai virus, Pneumonia virus of mice (PVM), rat coronavirus/ sialodacryoadenitis (RCV/SDA), Parvo virus (IFA Parvo), Kilham rat virus (KRV), CAR Bacillus, and Mycoplasma pulmonis (*M. Pul.*). In addition, fecal samples from representative animals will be externally examined for intestinal parasites.

2.4.5 Sentinels

After the selection of the study males, four of the remaining male rats will be randomly selected, eartagged, and designated as sentinels. They will be singly housed in the study room(s) with feed and water available *ad libitum* (as described below). They will be examined once daily by cageside observation for morbidity or mortality at the same time as the clinical observations or morbidity/mortality checks for the study animals. The clinical condition of sentinel animals will be recorded only in the event that an animal is moribund or found dead. If a sentinel animal is terminated moribund, blood will be collected at termination and serum samples frozen. During the male necropsies, the surviving sentinel males will be terminated, blood samples collected, and serum samples prepared. All sentinel serum samples will be submitted to BioReliance (Rockville, MD) for serological evaluation (see above section on Quality Control).

2.4.6 Quarantine

The male rats will be quarantined for approximately one week, with the prior concurrence of the RTI Animal Research Facility (ARF) veterinarian. They will be observed daily for general health status and ability to adapt to the ARF husbandry conditions. They will be released from quarantine, if suitable for use (based on QC results), by the attending ARF veterinarian or his designee.

2.5 ANIMAL HUSBANDRY

2.5.1 Housing, Feed, and Water

During the quarantine period, animals will be assigned to cages. Males will be singly housed in solid-bottom, polycarbonate cages (8"x19"x10.5") fitted with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Sani-Chip® cage bedding (P.J. Murphy, Forest Products, Inc., Montville, NJ) will be used in all cages. Pelleted feed (No. 5002 Purina Certified Rodent Chow®) and deionized water, produced at RTI from tap water from the Durham, NC water system, will be available *ad libitum* throughout quarantine and study periods. The water for the study animals is provided by an automatic watering system (Edstrom Industries, Inc., Waterford, WI). The analysis of the rodent chow for chemical composition and possible chemical contamination, and analysis of Durham City water will be provided by the suppliers and maintained in the study records. It is anticipated that contaminant levels will be below certified levels for both feed and water and will not affect the design, conduct, or conclusions of this study. In addition, each lot number of Purina 5002 feed used will be analyzed by the supplier for concentrations of the phytoestrogens genistein, daidzein, and glycitein. An aliquot of each lot number will be retained frozen for possible future analytical chemistry. The "metabolizable

energy content" of the feed (label value) will also be recorded and reported. Rat chow will be stored at approximately 60-70°F, and the period of use will not exceed six months from the milling date. At all times, animals will be housed, handled, and used according to the NRC Guide (NRC, 1996).

2.5.2 Environmental Conditions

Environmental conditions in the ARF will be continuously monitored, recorded, and controlled during the course of the study by an automated system (Siebe/Barber-Colman Network 8000 System) with Version 4.4.1 Signal® software (Siebe Environmental Controls (SEC)/ Barber-Colman Company, Loves Park, IL). Animal rooms used for this study will be maintained on a 14:10 hour light:dark cycle. Target conditions for temperature and relative humidity in the animal rooms will be between 64-79°F (18-26°C) and 30-70%, respectively, with 10-15 air changes per hour (NRC, 1996). Temperature and/or relative humidity excursions will be documented in the study records and the final report.

2.5.3 Animal Identification

All male rats will be individually identified by ear tag after arrival at RTI. In addition, all males assigned to the study will be given an animal study number. All data generated during the course of this study will be tracked by these numbers.

2.5.4 Limitation of Discomfort

Some toxicity may be caused by exposure at the high doses of each test material. Discomfort or injury to animals will be limited, in that if any animal becomes severely debilitated or moribund, it will be humanely terminated by CO_2 inhalation. All necropsies will be performed after terminal CO_2 asphyxiation.

3.0 EXPERIMENTAL DESIGN

3.1 STUDY DESIGN, TEST CHEMICALS, AND DOSE SELECTION

The study will be conducted in one component with staggered start dates, and will consist of four dose groups each of two chemicals, and a vehicle control group (total of 9 groups). Each group will be comprised of 15 males which have been randomized across treatment groups, based on body weight taken on SD -1 (the day before experimental start). The study males will be dosed by gavage once daily for 15 consecutive days. Table 1 presents the study design and target doses of the test chemicals. A graphical representation of the study design is presented in Figure 1 below.

The U.S. EPA selected two test chemicals for evaluation. The two test chemicals and their target/mechanism of action are as follows: (1) linuron (anti-androgen; competitive binding

to androgen receptor, and (2) methoxychlor (a xeno-estrogen through α-estrogen receptor, anti-estrogen through β-estrogen receptor and an anti-androgen through androgen receptor mediated mechanism). The doses for the present study were selected based on doses of these test chemicals used in a previously conducted pubertal assay (50 and 100 mg/kg/day linuron, 25 and 50 mg/kg/day methoxychlor). The high dose of each compound selected for the pubertal assay will be the high dose for the male *in vivo* study; the low dose in the pubertal assay will be the third highest dose in the *in vivo* male assay. The second highest dose will be halfway between the high and the third dose, and the lowest dose will be set below the third highest dose at approximately the same interval as that between the second and third doses.

Tentative Study Dates[a] (to be added to the protocol by amendment)

Males arrive at RTI:

Release of males from quarantine:

Dosing (Study Days [SD] 0-14):

Sacrifice of Males (SD 14):

Hormone Assays Complete:

Histopathology Complete

Data Tables to Study Director

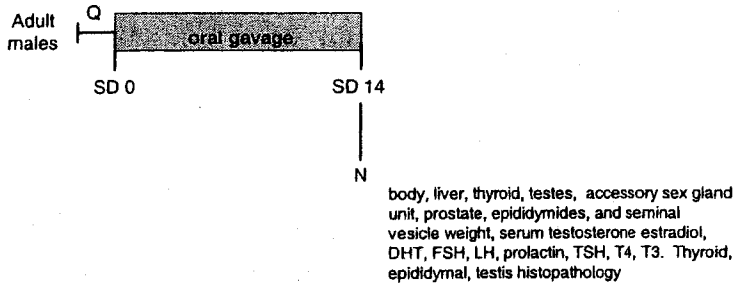
Internal Draft of the Report to RTI QAU:

Submission of audited draft final report:

Table 1. Study Design, Test Chemicals, and Target Doses

Group No.	No. Males	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
			COMPONENT 1		
1	15	–[a]	0	0.0	5
2	15	Linuron	25	5.0	5
3	15		50	10.0	5
4	15		75	15.0	5
5	15		100	20.0	5
6	15	Methoxychlor	12.5	2.5	5
7	15		25	5.0	5
8	15		37.5	7.5	5
9	15		50	10.0	5

[a] 0.25% aqueous methylcellulose, vehicle control



Key:
Q = quarantine
SD = study day
N = necropsy

Figure 1. Study Design for the 15-day Adult Male Assay

3.2 TREATMENT OF ADULT MALES

Beginning on SD 0, each male will be dosed with one of the test materials at one of the dose levels or the vehicle control (0.25 % aqueous methylcellulose). Each animal will be weighed daily on SD 0 to 13 prior to treatment, and the body weight recorded. Vehicle or dose formulations will be administered daily by oral gavage at a dosing volume of 5 ml/kg body weight from SD 0 for 15 consecutive days. Gavage dosing will use a 16-gauge, two-inch curved dosing needle fitted with a glass or plastic (disposable) tuberculin syringe of the appropriate volume for each treatment group. Animals will be dosed between 0700-1000 hours daily on SD 0-13. The treatments on SD 0-13 will be administered on a mg/kg body weight basis, adjusted based on the most recent body weight. On SD 14, the dose will be based on the body weight taken on SD 13. Dosing on SD 14 will take place prior to 1000 hours such that necropsy and

blood collection can be performed within 2 hours of dosing. The volume of the dose administered and the time of administration will be recorded each day.

3.3 OBSERVATION OF ADULT MALES

3.3.1 Clinical Observations

Clinical observations of male study animals will be documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours postdosing on SD 0-13. On SD 14, clinical observations will be made at dosing. The examining technicians will be unaware of the test materials or of dosage levels. Observations will be made for (but not limited to):

a. Any response with respect to body position, activity, coordination, or gait
b. Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
c. The presence of:
    1. Convulsions, tremors, or fasciculations
    2. Increased salivation
    3. Increased lacrimation or red-colored tears (chromodacryorrhea)
    4. Increased or decreased urination or defecation (including diarrhea)
    5. Piloerection
    6. Mydriasis or miosis (enlarged or constricted pupils)
    7. Unusual respirations (fast, slow, labored, audible, gasping, or retching)
    8. Vocalization

Cage-side examinations to detect moribund or dead rats will be conducted at least once daily throughout the study. Moribund rats will be sacrificed. Moribund and dead rats will be given a gross pathological evaluation. At every weighing, each rat will be individually handled and examined for abnormal behavior and appearance.

3.3.2 Male Body Weights and Feed Consumption

All study males will be weighed in the morning of SD -1 (prior to assignment to treatment groups), and every day in the morning on SD 0-13, for adjustment of dosing volume based on the most recent body weight. Males will also be weighed on SD 14, prior to necropsy. Male body weight and weight gains will be calculated and analyzed for SD 0-7 and 7-14. Feed weights for the individually-housed males will be recorded on SD 0, 7, and 14, and feed consumption will be reported as g/day and as g/kg body weight/day. From these determinations, as well as body weight data, mean food efficiency will be calculated for each group.

3.4 NECROPSY OF MALES

3.4.1 Gross Necropsy, Blood Collection, and Organ Weights

All rats found dead or sacrificed moribund prior to experimental start will be necropsied but tissues will not be saved or examined microscopically. After experimental start, all rats found dead or sacrificed moribund will be necropsied. Gross lesions and target organs (as described below) will be saved for optional histopathology. Blood will be taken at the time of necropsy. Rats sacrificed by design on SD 14 will be euthanized by CO_2 anesthesia within approximately two hours after the recorded time of dosing, and exsanguinated. Necropsies will take place between the hours of 0700 - 1000. For each animal, the maximum amount of blood obtainable (min. 15 ml.) will be collected via cardiac puncture for preparation of serum. Time of death and blood collection will be recorded for all animals.

Final body, liver, thyroid gland (fixed), testes, accessory sex gland unit, prostate, epididymides, and seminal vesicles (with fluid) weights will be taken. Mesenteric fat must be carefully removed with small surgical iris scissors from these tissues such that the fluid in the sex accessory glands is retained. All organs will be weighed to the nearest 0.1 mg. Relative organ weights (% of final body weight) will be calculated. Blood will be collected in a serum separator tube and placed on ice until serum is prepared.

3.4.2 Histology and Pathology

The liver and epididymides from each rat will be placed in formalin fixative, then embedded in paraffin. The thyroid glands and surrounding tissue will be removed and placed into formalin fixative for at least 48 hrs prior to trimming, weighing, and embedding in paraffin. Following fixation, final dissection of the thyroid will be performed by one individual in order to reduce the variability of the dissection procedure and hence, reduce the variability of the thyroid weights. Testes will be placed in Bouin's fixative for 24 hours after which they will be rinsed and stored in 70% alcohol until embedded in paraffin. The testes, epididymides, and thyroid will be evaluated microscopically. The embedded tissues will be sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E). Microscopic evaluations will be performed on control and high dose animals for all compounds. Only compounds which show effects in the high dose group will have the remaining groups evaluated. Stained sections will be evaluated by a Board Certified veterinary pathologist for pathologic abnormalities and potential treatment-related effects. Thyroids should be evaluated for morphologic changes such as altered follicular epithelial height, the relative number and staining characteristics of colloid, the extent of thyroid vascular supply, and the density, size, and shape of the thyroid follicles. The testes and epididymides will be evaluated for spermatogenesis, spermiogenesis, status of seminiferous tubules in the testis, and sperm in the epididymis, as well as the structural integrity of these organs. Liver will be evaluated microscopically at the discretion of the pathologist and the study director.

3.4.3 Hormone Analysis

Blood will be collected (target minimum 10 ml) at the time of sacrifice from all animals. The blood will be placed in a serum separator tube on ice until the serum is prepared. The blood will be allowed to clot and centrifuged under refrigeration at a setting of approximately 1400 x g for approximately ten minutes. Serum will be stored between -65 °C and -85°C until analyzed. Serum testosterone (T), estradiol (E2), dihydrotestosterone (DHT), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PL), thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) levels will be measured by commercially available radioimmunoassays (RIAs). If serum is limiting, priority of analysis will be T, E2, FSH, LH, TSH, T4, T3, PL, and DHT. Any remaining serum will be discarded after the report is issued.

4.0 STATISTICAL ANALYSES

All data for a single chemical and concurrent vehicle control group (body weights and weight gains, body and organ weights at necropsy, food consumption, and serum hormones) will be analyzed using either parametric analysis of variance (ANOVA) under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967) which do not assume homogeneity of variance or normality. The homogeneity of variance assumption will be examined via Levene's test (Levene, 1960). If Levene's test indicates lack of homogeneity of variance ($p<0.05$), robust regression methods will be used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They will be used to test for linear trends across dose as well as overall treatment group differences (via Wald chi-square tests). Significant overall treatment effects will be followed by single degree-of-freedom *t*-tests for exposed vs. control group comparisons, if the overall treatment effect is significant. If Levene's test does not reject the hypothesis of homogeneous variances, standard ANOVA techniques will be applied for comparing the treatment groups. The GLM procedure in SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) will be used to test for linear trend, evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS®, and the robust regression methods are available in the REGRESS procedure of SUDAAN® Release 8.0 (RTI, 2001). Organ weights will also be analyzed by Analysis of Covariance (ANCOVA) using the body weight at necropsy as the covariate. When statistically significant effects are observed, treatment means will be examined further using LSMeans.

The unit of comparison will be the male rat on study. A test for statistical outliers will be performed in the UNIVARIATE procedure of SAS® Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) on male body and organ weights and feed consumption. If examination of pertinent study data do not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data will be excluded from summarization and analysis and will be designated as outliers. For all statistical tests, $p \leq 0.05$ (one- or two-tailed) will be used as the criterion for significance.

5.0 RETENTION OF SPECIMENS AND RECORDS

All specimens and records which remain the responsibility of RTI will be retained in the RTI archives for two years at the performing laboratory's expense. Beyond two years, continued retention will be at additional cost to the Sponsor.

6.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study.

7.0 REPORTING

7.1 STATUS REPORTS

Status reports will be provided to the EDSP Program Manager.

7.2 DRAFT AND FINAL REPORTS

A draft report will be submitted to the Sponsor's Representative within three months after completion of the data collection (including hormone analysis and pathology).

The final report will include:

- Abstract
- Objectives
- Materials and Methods
- Results
- Discussion
- Conclusions
- References
- Summary in-life and necropsy data with statistical analyses
- Individual animal data: in-life and necropsy
- Protocol, any amendments, or any deviations from the protocol
- QAPP, any amendments, or any deviations from the QAPP
- Histopathology report

Individual Data Male Rats

a. Identification number
b. Clinical signs
c. Body weight daily, feed on Day 0, 7, and 14
d. Age at death and manner of death
e. Weight at necropsy
f. Organ weights
g. Gross Necropsy Observations
h. Serum Hormone levels

Summary of Data From Male Rats

a. Mean periodic body weights and weight gains
b. Feed consumption
c. Clinical signs
d. Organ weights
e. Histopathological Data
f. Serum Hormone Levels

8.0 PERSONNEL

Study Director:	Carol D. Sloan, M.S.
Work Assignment Leader:	Julia D. George, Ph.D.
Principal Investigator:	Rochelle W. Tyl, Ph.D., DABT
ARF Veterinarian:	Donald B. Feldman, D.V.M., ACLAM
ARF Manager:	Frank N. Ali, M.B.A., RLATG, ILAM
Laboratory Supervisor:	Melissa C. Marr, B.A., RLATG
Data Analyst and Reproductive Toxicity Supervisor:	Christina B. Myers, M.S.

Statistical Advisor:	Gayle S. Bieler, M.S.
Research Assistant, Data Entry:	Timothy W. Wiley, B.S.
Research Biologist:	William P. Ross, B.A.
Biologists:	Vickie I. Wilson Lawson B. Pelletier, RVMT, LAT
Biological Laboratory Assistants:	Malcolm D. Crews, ALAT Robin T. Krebs, ALAS
Endocrinology:	Patricia A. Fail, Ph.D. Carol S. Sloan, M.S. Kristi D. Vick, B.S. Angela Parham, B.S. Susan Pearce, B.S. Amanda Goodman, B.S.
Quality Assurance:	Doris J. Smith, B.S., Manager Celia D. Keller, M.S. Patricia D. Hall Marcia D. Phillips, M.S. D. Denise Rowe, M.L.S. Tiffany M. Kenney, B.S. Erica D. Shinuald, B.S. Jennifer E. Jones, B.S. Jacques Hargett, B.S.
Histology:	EPL, Inc.
Pathology:	John C. Seely, D.V.M., ACVP (EPL, Inc.)

Additional study team members to be determined.

9.0 STUDY RECORDS TO BE MAINTAINED

Protocol and any Amendments

QAPP and any Amendments

List of any Protocol Deviations

List of QAPP Deviations

List of Standard Operating Procedures

Animal Requisition and Receipt Records

Quarantine Records

Temperature and Humidity Records for the Animal Room(s)

Animal Research Facility Room Log(s)

Durham City Water Analysis (analyzed monthly, reported annually)

Feed Type, Source, Lot Number, Dates Used, Certification, Analytical Results

Dosage Code Records Containing Five-Digit Rx Code, Color Code, and Concentration

Dose Formulation Receipt and Use Records

Male Distribution into Groups

Male Dosing Forms

Body Weights

Clinical Signs

Food Weights

Male Necropsy Records: Body weight, organ weights, gross observations, required (and optional, if done) organ histopathology

Statistical Analysis Records

Histopathology Report

Serum Thyroid Hormone Analyses (Serum testosterone, estradiol, DHT, FSH, LH, prolactin, TSH, T4, and T3 levels)

Correspondence

10.0 REFERENCES

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O'Connor, J.C., J.C. Cook, S.C. Craven, C.S. Van Pelt, and J.P. Obourn (1996). An *in vivo* battery for identifying endocrine modulators that are estrogenic or dopamine regulators. *Fundam. Appl. Toxicol.* **33**, 182-195.

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Zeger, S. and K. Liang (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* **42**, 121-130.

ATTACHMENT

Material Safety Data Sheets (MSDSs)

Linuron	CAS No. 330-55-2
Methoxychlor	CAS No. 72-43-5

Chem Service, Inc. PS-372
MATERIAL SAFETY DATA SHEET

Invoice: CS231745 PO: 11136807EAC

Printed: 08/26/2002
Last Revised: July 6, 2001

SECTION 1 - CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Catalog Number: PS-372
Description: Linuron
Other Name(s): 3-[3.4-Dichlorophenyl]-1-methoxy-1-methylurea

Supplied by CHEM SERVICE, Inc. PO BOX 599, WEST CHESTER, PA 19381 (610)-692-3026
EMERGENCY PHONE: 1-610-692-3026

SECTION 2 - COMPOSITION, INFORMATION ON INGREDIENTS

CAS No.: 330-55-2
Description: Linuron
EINECS No.: 206-356-5
Hazard Symbols: Xn

SECTION 3 - HAZARDS IDENTIFICATION

Contact lenses should not be worn in the laboratory.
All chemicals should be considered hazardous - Avoid direct physical contact!
Can cause eye irritation. Can cause skin irritation.
Dust and/or vapors can cause irritation to respiratory tract.
Can be irritating to mucous membranes. May be harmful if absorbed through the skin.
May be harmful if inhaled. May be harmful if swallowed.

SECTION 4 - FIRST AID MEASURES

An antidote is a substance intended to counteract the effect of a poison. It should be administered only by a physician or trained emergency personnel. Medical advice can be obtained from a POISON CONTROL CENTER.

In case of contact: Flush eyes continuously with water for 15-20 minutes. Flush skin with water for 15-20 minutes. If no burns have occurred-use soap and water to cleanse skin.
If inhaled remove patient to fresh air. Administer oxygen if patient is having difficulty breathing. If patient has stopped breathing administer artificial respirations.
If patient is in cardiac arrest administer CPR.
Continue life supporting measures until medical assistance has arrived.
Remove and wash contaminated clothing.
If patient is exhibiting signs of shock - Keep warm and quiet.
Contact Poison Control Center immediately if necessary. Induce vomiting if swallowed.
Do not administer liquids or induce vomiting to an unconscious or convulsing person.
If patient is vomiting-watch closely to make sure airway does not become obstructed by vomit.
Get medical attention if necessary.

SECTION 5 - FIRE AND EXPLOSION DATA

Flash Point: Not Available .

SECTION 5 - FIRE AND EXPLOSION DATA CONTINUED

Extinguishing Media:
Carbon dioxide, dry chemical powder or spray.
Upper Explosion Limit: Not Available
Lower Explosion Limit: Not Available
Autoignition Temperature: Not Available
NFPA Hazard Rating: Not Available

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Spills or leaks: Evacuate area. Wear appropriate OSHA regulated equipment. Ventilate area.
Sweep up and place in an appropriate container. Hold for disposal.
Wash contaminated surfaces to remove any residues.
Remove contaminated cloting and wash befire reuse.

SECTION 7 - HANDLING AND STORAGE

Handling:
This chemical should be handled only in a hood. Eye shields should be worn.
Use appropriate OSHA/MSHA approved safety equipment.
Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation
Wash thoroughly after handling.

Storage:
Store in a cool dry place. Store only with compatible chemicals.
Keep tightly closed.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

OSHA PEL (TWA): Not Available
ACGIH TLV (TWA): Not Available
ACGIH TLV (STEL): Not Available

Personal Protective Equipment
Eyes: Wear Safety Glasses.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to minimize contact with skin.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 requirements must be followed whenever workplace conditions warrant a respirator's use.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Color:	Colorless
Phase:	Crystalline solid
Melting Point:	93-94 C
Boiling Point:	Not Available
Specific Gravity:	Not Available
Vapor Pressure:	Not Available
Vapor Density:	Not Available

Solubility in Water:	Not Available
Odor:	Not Available
Evaporation Rate (Butyl acetate=1):	Not Available
Molecular Weight	249.11
Molecular Formula	C9H10C12N2O2

SECTION 10 - STABILITY AND REACTIVITY

Sensitive to light - dark color does not affect purity. Sensitive to heat. Decomposes under alkaline conditions. Decomposes under acidic conditions.

SECTION 11 - TOXICOLOGY INFORMATION

RTECS: YS9100000
Oral Rat or Mouse LD50: 4000mg/kg
Dermal Rat or Mouse LD50: >2500
Rat or Mouse LC50: Not Available

Carcinogenicity
OSHA: No
IARC: No
NTP: No
ACGIH: No
NIOSH: No
Other: No

SECTION 12 - ECOLOGICAL INFORMATION

Ecotoxicity: Not Available
Environmental Fate: Not Available

SECTION 13 - DISPOSAL CONSIDERATIONS

DISPOSAL: Burn in a chemicals incinerator equipped with an afterburner and scrubber.

SECTION 14 - TRANSPORTATION INFORMATION

Not regulated as a hazardous material.

SECTION 15 - REGULATORY INFORMATION

European Labeling in Accordance with EC Directives
Hazard Symbols: Xn
Risk Phrases
R40
Possible risk of irreversible effects.

Safety Phrases
S36/37
Wear suitable protective clothing and gloves.

SECTION 16 - OTHER INFORMATION

The above information is believed to be correct on the date it is published and must not be considered all inclusive. The information has been obtained only by a search of available literature and is only a guide for handling the chemicals. OSHA regulations require that if other hazards become evident, an upgraded MSDS must be made avalible to the employee within three months. RESPONSIBILITY for updates lies with the employer and not with CHEM SERVICE, Inc.

Persons not specifically and properly trained should not handle this chemical or its container. This MSDS is provided without any warranty expressed or implied, including merchantability or fitness for any particular purpose.

This product is furnished FOR LABORATORY USE ONLY! Our products may NOT BE USED as drugs, cosmetics, agricultural or pesticidal products, food additives or as household chemicals.

CHEM SERVICE -- F910 METHOXYCHLOR
MATERIAL SAFETY DATA SHEET
NSN: 655000F051063
Manufacturer's CAGE: 8Y898
Part No. Indicator: A
Part Number/Trade Name: F910 METHOXYCHLOR

General Information

Company's Name: CHEM SERVICE INC
Company's Street: 660 TOWER LN
Company's P. O. Box: 3108
Company's City: WEST CHESTER
Company's State: PA
Company's Country: US
Company's Zip Code: 19381-3108
Company's Emerg Ph #: 215-386-2100/215-692-3026
Company's Info Ph #: 215-692-3026/800-452-9994
Record No. For Safety Entry: 001
Tot Safety Entries This Stk#: 001
Status: SE
Date MSDS Prepared: 25JAN95
Safety Data Review Date: 19SEP96
Preparer's Company: CHEM SERVICE INC
Preparer's St Or P. O. Box: 660 TOWER LN
Preparer's City: WEST CHESTER
Preparer's State: PA
Preparer's Zip Code: 19381-3108
MSDS Serial Number: CCDMW

Ingredients/Identity Information

Proprietary: NO
Ingredient: METHOXYCHLOR (IARC CARCINOGEN - GROUP 3) *96-3*
Ingredient Sequence Number: 01
NIOSH (RTECS) Number: KJ3675000
CAS Number: 72-43-5
OSHA PEL: 15 MG/CUM
ACGIH TLV: 10 MG/CUM

Physical/Chemical Characteristics

Appearance And Odor: COLORLESS CRYSTALLINE SOLID W/FRUITY/PLEASANT ODOR
Melting Point: 186.8-192F
Solubility In Water: INSOLUBLE

Fire and Explosion Hazard Data

Extinguishing Media: CO2, DRY CHEMICAL POWDER/SPRAY

Reactivity Data

Stability: YES
Materials To Avoid: STRONG OXIDIZING AGENTS
Hazardous Poly Occur: NO

Health Hazard Data

LD50-LC50 Mixture: ORAL LD50(RAT): 6000 MG/KG
Route Of Entry - Inhalation: YES
Route Of Entry - Skin: YES
Route Of Entry - Ingestion: YES
Health Haz Acute And Chronic: SKIN/EYES: CAN CAUSE IRRITATION. CAN BE IRRITATING TO MUCOUS MEMBRANES. MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN, INHALED/IF SWALLOWED. EXPOSURE CAN CAUSE KIDNEY/LIVER DAMAGE.
Carcinogenicity - NTP: NO
Carcinogenicity - IARC: NO
Carcinogenicity - OSHA: NO
Explanation Carcinogenicity: NONE
Signs/Symptoms Of Overexp: IRRITATION
IF NO BURNS HAVE OCCURRED, CLEANSE W/SOAP & WATER. INHALATION: REMOVE TO FRESH AIR. GIVE OXYGEN/CPR IF NEEDED. IF IN SHOCK, KEEP WARM/QUIET. INGESTION: INDUCE VOMITING. DON'T GIVE LIQUIDS/INDUCE VOMITING IF UNCONSCIOUS/CONVULSING. IF VOMITING, WATCH CLOSELY TO MAKE SURE AIRWAY DOESN'T BECOME OBSTRUCTED BY VOMIT. OBTAIN MEDICAL ATTENTION IN ALL CASES.

Precautions for Safe Handling and Use

Steps If Matl Released/Spill: EVACUATE AREA. WEAR OSHA REGULATED EQUIPMENT. VENTILATE AREA. SWEEP UP & PLACE IN AN APPROPRIATE CONTAINER. HOLD FOR DISPOSAL. WASH CONTAMINATED SURFACES TO REMOVE ANY RESIDUES.
Waste Disposal Method: BURN IN A CHEMICALS INCINERATOR EQUIPPED W/AN AFTERBURNER & SCRUBBER/DISPOSE OF IN ACCORDANCE W/LOCAL, STATE & FEDERAL REGULATIONS.
Precautions-Handling/Storing: KEEP TIGHTLY CLOSED. STORE IN A COOL, DRY PLACE. STORE ONLY W/COMPATIBLE CHEMICALS. THIS PRODUCT IS FURNISHED FOR LABORATORY USE ONLY.
Other Precautions: AVOID CONTACT W/SKIN, EYES & CLOTHING. DON'T BREATHE VAPORS. PRODUCT MAY NOT BE USED AS DRUGS, COSMETICS, AGRICULTURAL/ PESTICIDAL PRODUCTS, FOOD ADDITIVES/AS HOUSEHOLD CHEMICALS.

Control Measures

Respiratory Protection: USE APPROPRIATE OSHA/MSHA APPROVED SAFETY EQUIPMENT.
Ventilation: THIS CHEMICAL SHOULD BE HANDLED ONLY IN A HOOD.

Eye Protection: EYE SHIELDS
Work Hygienic Practices: REMOVE/LAUNDER CONTAMINATED CLOTHING BEFORE REUSE. CONTACT LENSES SHOULDN'T BE WORN IN THE LABORATORY.
Suppl. Safety & Health Data: PERSONS NOT SPECIFICALLY/PROPERLY TRAINED SHOULDN'T HANDLE THIS CHEMICAL/ITS CONTAINER. ALL CHEMICALS SHOULD BE CONSIDERED HAZARDOUS-AVOID DIRECT PHYSICAL CONTACT. DATA INFORMATION IS FOR ACETONE.

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Transportation Data

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Disposal Data

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Label Data

==========

EPA Contract No.: 68-W-01-023 (Battelle Prime Contractor)
EPA Work Assignment No.: WA-28
RTI Contract No.: 65U-08055.001.021
RTI Study Code: Rt03-ED07
RTI Master Protocol No.: RTI-871

Amendment 1

TITLE: 15-Day Tier 1 Screen of Endocrine Active Compounds Administered by Gavage to Adult Male Sprague-Dawley (CD®) Rats

SPONSOR: Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

TESTING FACILITY: RTI International
Chemistry and Life Sciences
Center for Life Sciences and Toxicology
3040 Cornwallis Road Post Office Box 12194
Research Triangle Park, NC 27709

PROTOCOL Amendment 1	RTI International P.O. Box 12194 Research Triangle Park, NC 27709	RTI-871 Page 2 of 15

APPROVED BY:

Rochelle W. Tyl, Ph.D., DABT — Date 02/13/04
Project Toxicologist
Endocrine Disruptor Screening Program
RTI International

Carol D. Sloan, M.S., LATG — Date 2-13-04
Study Director
Endocrine Disruptor Screening Program
RTI International

Gary Timm — Date 2-17-04
Work Assignment Manager
Endocrine Disruptor Screening Program
U.S. EPA

Julia D. George, Ph.D. — Date 2/13/04
Work Assignment Leader, WA 2-28
Endocrine Disruptor Screening Program
RTI International

L. Greg Schweer, MS — Date 2-17-04
Project Officer
Endocrine Disruptor Screening Program
U.S. EPA

David P. Houchens, Ph.D. — Date 2/13/04
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

REVIEWED BY:

Marcia D. Phillip, M.S. — Date 2-13-2004
Quality Assurance Specialist
RTI International

Terri Pollock, B.A. — Date 2-13-04
Quality Assurance Manager
Battelle Memorial Institute

Item 1

Cover Page, Proposed Experimental Dates, which read:

"PROPOSED EXPERIMENTAL START DATE: March 2003
PROPOSED EXPERIMENTAL TERMINATION DATE: July 2003"

is hereby amended to read:

"PROPOSED EXPERIMENTAL START DATE: **May 2003**
PROPOSED EXPERIMENTAL TERMINATION DATE: **September 2003"**

Justification: The start of the study was rescheduled in accordance with the development of the analytical chemistry methods and the flow of laboratory work.

Item 2

Table of Contents, page 3, 2.1.3 Methylcellulose which reads:

Page 8

Is hereby amended to read:

Page 7

Justification: The page was incorrectly listed in the TOC in the Protocol

Item 3

Section 1.0 , page 5, paragraph which reads:

Based on the EDSTAC's recommendations, one of the assays that the EPA has proposed to validate as a potential alternative for other assays in the Tier 1 battery in an endocrine disruptor screening program is a male *in vivo* assay (see FR Vol. 63, No. 248, pp. 71541-71568, December 28, 1998). The utility of this battery for screening unknown compounds for endocrine activity will be evaluated.

Is hereby amended to read:

Based on the EDSTAC's recommendations, one of the assays that the EPA has proposed to validate as a potential alternative for other assays in the Tier 1 battery in an endocrine disruptor screening program is a male *in vivo* assay (see FR Vol. 63, No. 248, pp. 71542-71568, December 28, 1998). The utility of this battery for screening unknown compounds for endocrine activity will be evaluated.

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Justification:

Wrong page number was used initially.

Item 4

Section 2.1.1, page 6 that reads :

Supplier:	Chem Services
Lot Number:	273-81B
Purity:	99%
Appearance:	Crystalline solid
Molecular Formula:	C9H10Cl2N2O2
Molecular Weight:	249
Storage, Bulk Chemical:	Room Temperature
Storage, Test Solution:	4 deg C

Is Hereby Amended to read:

Supplier:	**Chem Services, West Chester, PA**
Lot Number:	**301-91A**
Purity:	99%
Appearance:	Crystalline solid
Molecular Formula:	C9H10Cl2N2O2
Molecular Weight:	249
Storage, Bulk Chemical:	Room Temperature
Storage, Test Solution:	4 deg C

Justification:
To include the city and state of the supplier of linuron. To change the lot number of linuron that was used in the study.

Item 5

Section 2.1.2, page 7, which reads:

Supplier:	Sigma
Lot Number:	49H1328
Purity:	95.2%
Appearance:	Light orange powder
Molecular Formula:	C16H15Cl3O2
Molecular Weight:	345.7
Storage, Bulk Chemical:	Room Temperature
Storage, Test Solution:	4 deg C

Is hereby amended to read:

Supplier: **Sigma Chemical Company, St. Louis, Missouri**
Lot Number: 49H1328
Purity: 95.2%
Appearance: Light orange powder
Molecular Formula: C16H15Cl3O2
Molecular Weight: 345.7
Storage, Bulk Chemical: Room Temperature
Storage, Test Solution: 4 deg C

Justification:
To include the city and state of the supplier of methoxychlor.

Item 6

Section 2.1.3, Vehicle: Methylcellulose, page 7, which reads in part:

"Supplier: (to be added by sponsor)
Lot Number: (to be added by sponsor)
Purity: (to be added by sponsor)
Appearance: (to be added by sponsor)
Molecular Formula: $(C_7H_{14}O_5)_x$ (polymer)

Molecular Weight: 40,000 to 180,000 (polymer)
Storage, Bulk Chemical: (to be added by sponsor)
Storage, Vehicle Solution: (to be added by sponsor)"

is hereby amended to read:

"Supplier: **Sigma Chemical Company, #M-0512**
Lot Number: **062K0144**
Purity: **None given**
Appearance: **white powder**
Molecular Formula: $(C_7H_{14}O_5)_x$ (polymer)

Molecular Weight: 40,000 to 180,000 (polymer)
Storage, Bulk Chemical: **dry powder stored at room temperature**
Storage, Vehicle Solution: **4° C.**"

Justification: Addition of chemical information that was not available when the protocol was written.

Item 7

Section 2.3, Dose Formulation and Analysis, page 8, which reads:

The dosing formulations will be prepared at a frequency determined by stability tests initiated prior to the start of the study. Formulations will be prepared at the Battelle Chemical Repository, Sequim, WA, and stored in wide-mouth, 200 ml amber bottles. They will be shipped on ice or with frozen cold packs via 24-hour express delivery and logged into the RTI Materials Handling Facility. Prior to transfer to the Reproductive and Developmental Toxicology Laboratory for dosing, the concentration of the dose formulations will be verified by the Battelle Chemical Respository. The test materials will be mixed in 0.25% aqueous methylcellulose, with the concentration determined by the following formula:

$$\text{Concentration (mg / ml)} = \frac{\text{Dose per time (mg / kg)}}{\text{Dosage volume per time (5 ml / kg)}}$$

An aliquot of each dose level per formulation will be analyzed by Battelle. The dosing bottles will be identified at RTI by a five-digit random number Rx code, and a color code. Personnel, other than the Laboratory Supervisor, Project Toxicologist, and Study Director, will not be informed of the test chemicals or formulation concentrations until all laboratory work is completed (i.e., the study technicians will be "blind" for chemical and dose). The dosing formulations will be stored at refrigerator temperatures. Prior to dosing each day, the formulations will be removed from the refrigerator. Solutions will be shaken and warmed to room temperature. Aliquots (at least 5 ml) from the dosing bottles will be collected on the first day of dosing (SD 0), the eighth day of dosing (SD 7) and the last day of dosing (SD 14). These samples will be held in refrigerated storage until the end of the dosing period, when they will be shipped overnight on frozen cold packs to Battelle Chemical Repository, Sequim, WA, for analysis."

is hereby amended to read:

Prior to initiation of treatment, the bulk chemicals and the methylcellulose will be characterized by the sponsor at the Battelle Chemical Repository, Sequim, WA. In addition, Battelle, Sequim will verify dose formulation and handling procedures for each chemical formulated at each of the specified doses. Samples of the low dose formulation of each chemical will be analyzed for homogeneity in addition to chemical concentration. Verification of dose formulation and handling procedures requires that all assay results fall within 90 to 110% of the target concentrations, with a coefficient of variation of ≤5% for samples taken from the same nominal concentration. The bulk chemicals and vehicle will be shipped from Battelle, Sequim, on ice or with frozen cold packs via 24-hour express delivery, to RTI International and logged into the RTI Materials Handling Facility (MHF). Dose formulation will take place in the RTI MHF.

The dosing formulations will be prepared weekly. Formulations will be prepared not more that three days prior to first administration, and will be administered to the animals for no more than 7 consecutive days. Thus, three sets of formulations will be used for this study. Linuron or

methoxychlor will be formulated in 0.25% aqueous methylcellulose. Dose formulations will be prepared according to the following formula:

$$\text{Concentration (mg / ml)} = \frac{\text{Dose per time (mg / kg)}}{\text{Dosage volume per time (5 ml / kg)}}$$

Dose formulations will be labeled with the mix date, study code (Rt03-ED07), concentration codes (random five-digit code and color code), storage conditions, and expiration date. Both the numerical and color codes will appear on individual animal cage cards and dosing records; other animal records will contain only the five-digit code. Except for the laboratory supervisor, staff members involved with the in-life and postmortem evaluation of animals will not be informed of the concentrations associated with each code until all laboratory work has been completed.

The 0.25% methylcellulose will be prepared the following RTI Materials Handling Facility (MHF) method:

Preparation Of 0.25% Methlycellulose Vehicle

TOTAL VOLUME = 3000 ml

Divide the total amount of deionized distilled water needed approximately in half. Cool one of the portions in the refrigerator. Use the remaining water to begin the vehicle preparation.

Calibrate a glass beaker to 3000 ml with a magnetic stir bar in place. Heat ~1000 ml of room temperature water to boiling in the calibrated beaker on a magnetic stirrer/hot plate. While the water is heating weigh 7.5 g of 4000 centipoise methylcellulose into a small glass beaker.

Once the water begins to boil turn the stirplate heater off. When the water stops boiling, add the methylcellulose to the water while stirring rapidly. Stir until the methylcellulose is thoroughly dispersed.

Cover the beaker with aluminum foil and slow the stirring so the vehicle does not foam. Stir for ~30 minutes. Use the remaining room temperature water to rinse the residual foam from the sides of the beaker. Stir for an additional 15 minutes.

While stirring, begin adding the refrigerated water in ~500 ml increments to the vehicle. Stir for ~5 minutes between additions. Continue adding the cool water in increments until it reaches the calibration line. As the vehicle cools to room temperature it will begin to clear. If the vehicle has not cooled sufficiently, place the beaker in an ice bath on a stir plate and continue to stir. Add water to the calibration line and stir for ~5 minutes.

Repeat Steps 1 thru 5 for each additional 3L batch if needed.

Transfer to amber bottles or plastic carboys and refrigerate. Warm the vehicle to room temperature prior to use in formulations.

The methoxychlor formulations will be adjusted to account for the 95% purity by adding 105% of the target amount (by weight) of methoxychlor. The formulations will be prepared in an calibrated clear glass beaker.

Duplicate 1 ml samples for homogeneity confirmation will be collected from the top, middle and bottom of the low and high dose of both Linuron and Methoxychlor formulations on the first day of formulation. Duplicate 1 ml samples for dose confirmation will be collected from the.middle of the 2 mid does of each test chemical and control on the first formulation day and from all doses for the subsequent formulation days. All samples will be collected using a 16 gauge gavage needle attached to a 3 ml disposable syringe. Samples will be collected while the suspensions are stirred.

These aliquots will be placed in individually labeled glass scintillation vials. One set of samples will be shipped, on frozen cold packs via 24-hour express delivery, to Battelle, Sequim for analysis. The other set of samples will be stored at approximately -20°C and maintained until they are needed for analysis or discarded after 90 days, whichever comes first.

The dose formulations will be stored at ~5° C. Approximately one hour before dosing, the jar will be placed on a stirrer and mixed at room temperature. The formulations will be stirred throughout the dosing each day. Additional duplicate analytical samples (one per formulation) will be taken as described above on the first and last day of dosing for each formulation, and shipped to Battelle, Sequim, for analysis."

Justification: Due to the requirement that formulations be mixed not more than three days prior to dosing, and used for not more than 7 consecutive days of dosing, the formulations were made at RTI. Effective April 14, 2003, a subcontract between RTI and Battelle was put into place to authorize this activity. Item 7 provides the correct dose formulation procedures.

Item 8

Section 3.1, Study Design, Test Chemicals, and Dose Selection, page 12, Tentative Study Dates:, which reads:

Tentative Study Dates[a] (to be added to the protocol by amendment)

Males arrive at RTI:

Release of males from quarantine:

Dosing (Study Days [SD] 0-14):

Sacrifice of Males (SD 14):

Hormone Assays Complete:

Histopathology Complete

Data Tables to Study Director

Internal Draft of the Report to RTI QAU:

Submission of audited draft final report:

is hereby amended to read:

Tentative Study Dates

Males arrive at RTI:	**May 12, 2003**
Release of males from quarantine:	**May 19, 2003**
Dosing (Study Days [SD] 0-14):	**May 19 - June 6, 2003**
Sacrifice of Males (SD 14):	**June 2- June 6, 2003**
Hormone Assays Complete:	**September 19, 2003**
Histopathology Complete:	**July 18, 2003**
Data Tables to Study Director	**October 17, 2003**
Notebooks to QA	**October 17, 2003**
Internal Draft of the Report to RTI QAU:	**January 27, 2004**
Submission of audited draft final report:	**February 29, 2004**

Justification: Inclusion of the study schedule, which was not available when the protocol was written

Item

Section 3.3.1, Clinical Observations, page 14, which reads:

Cage-site examinations to detect moribund or dead rats will be conducted at least once daily throughout the study. Moribund rats will be sacrificed. Moribund and dead rats will be given a gross pathological evaluation. At every weighing, each rat will be individually handled and examined for abnormal behavior and appearance.

Is hereby amended to read:

Cage-site examinations to detect moribund or dead rats will be conducted at least once daily throughout the study. **Moribund rats will be euthanized by CO_2 anesthesia**.. Moribund and dead rats will be given a gross pathological evaluation. At every weighing, each rat will be individually handled and examined for abnormal behavior and appearance.

Justification: To explain the method of sacrifice for moribund rats.

Item 10

Section 3.3.1, Clinical Observations, which reads:

Clinical observations of male study animals will be documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours postdosing on SD 1-14. On SD 15, clinical observations will be made at dosing. The examining technicians will be unaware of the test materials or of dosage levels. Observations will be made for (but not limited to):

Is hereby amended to read:

Clinical observations of male study animals will be documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours postdosing on SD 1-14. On SD 15, clinical observations will be made at dosing. **The examining technicians will be aware of the test materials but not the dosage levels**. Observations will be made for (but not limited to):

Justification: The technicians will need to read the protocol and therefore will know the test materials to be used but will be unaware of the chemical and dosage level while dosing.

Item 11

Section 3.4.2, Histology and Pathology, page 15, which reads:

"The liver and epididymides from each rat will be placed in formalin fixative, then embedded in paraffin. The thyroid glands and surrounding tissue will be removed and placed into formalin fixative for at least 48 hrs prior to trimming, weighing, and embedding in paraffin. Following fixation, final dissection of the thyroid will be performed by one individual in order to reduce the variability of the dissection procedure and hence, reduce the variability of the thyroid weights. Testes will be placed in Bouin's fixative for 24 hours after which they will be rinsed and stored in 70% alcohol until embedded in paraffin. The testes, epididymides, and thyroid will be evaluated microscopically. The embedded tissues will be sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E). Microscopic evaluations will be performed on control and high dose animals for all compounds. Only compounds which show effects in the high dose group will have the remaining groups evaluated. Stained sections will be evaluated by a Board Certified veterinary pathologist for pathologic abnormalities and potential treatment-related effects. Thyroids should be evaluated for morphologic changes such as altered follicular epithelial height, the relative number and staining characteristics of colloid, the extent of thyroid vascular supply, and the density, size, and shape of the thyroid follicles. The testes and epididymides will be evaluated for spermatogenesis, spermiogenesis, status of seminiferous tubules in the testis, and sperm in the epididymis, as well as the structural integrity of these organs. Liver will be evaluated microscopically at the discretion of the pathologist and the study director.

Is hereby amended to read:

"Tissues taken at necropsy will be placed in fixative and then transferred to Experimental Pathology Laboratories (EPL) for processing. The thyroid (with trachea attached), liver and one

epididymides from each rat will be placed in formalin fixative. Testes will be placed in Bouin's fixative for 24 hours after which they will be rinsed and stored in 70% alcohol. The thyroid will be weighed at EPL, after removal of the trachea. The tissues will then be embedded in paraffin, sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E) for subsequent histological evaluations. The testes, epididymides, and thyroid will be evaluated microscopically. Microscopic evaluations will be performed on control and high dose animals for all compounds. If significant adverse effects are observed in the high dose group, the remaining treated groups will be evaluated. Stained sections will be evaluated by a Board Certified veterinary pathologist for pathologic abnormalities and potential treatment-related effects. Thyroids should be evaluated for morphologic changes such as altered follicular epithelial height, the relative number and staining characteristics of colloid, the extent of thyroid vascular supply, and the density, size, and shape of the thyroid follicles. The testes and epididymides will be evaluated for spermatogenesis, spermiogenesis, status of seminiferous tubules in the testis, and sperm in the epididymis, as well as the structural integrity of these organs. Liver will be evaluated microscopically at the discretion of the pathologist and the study director."

Justification: Clarification of changes in histology and pathology procedures resulting from designating EPL as the histology laboratory (closing of RTI Histology Laboratory effective 07/01/02).

Item 12

Section 3.4.3, page 16, Hormone Analysis which reads:

Blood will be collected (target minimum 10 ml) at the time of sacrifice from all animals. The blood will be placed in a serum separator tube on ice until the serum is prepared. The blood will be allowed to clot and centrifuged under refrigeration at approximately 1400 x g for approximately ten minutes. Serum will be stored between -65 °C and -85°C until analyzed. Serum testosterone, estradiol, DHT, FSH, LH, prolactin, TSH, T4, and T3 levels will be measured by commercially available radioimmunoassays (RIAs). If serum is limiting, priority of analysis will be determined by the study director. Any remaining serum will be discarded after the report is issued.

Is hereby amended to read:

Blood will be collected (targrt minimum 10 ml) at the time of sacrifice from all animals. The blood will be placed in a serum separator tube on ice until the serum is prepared. The blood will be allowed to clot and centrifuged under refrigeration at approximately 1400 x g for approximately ten minutes. Serum will be stored between -65 °C and -85°C until analyzed. Serum testosterone, estradiol, DHT, FSH, LH, prolactin, TSH, T4, and T3 levels will be measured by commercially available radioimmunoassays (RIAs). If serum is limiting, priority of analysis will be determined by the study director. **[Any remaining serum will be discarded after the report is issued.]**

Justification:Removal of the statement to discard the serum, it will be kept for two years as the other specimens.

Item 13

Section 8.0 Personnel, page 19l which reads:

Histology: EPL, Inc.

Is hereby amended to read:

Histology: EPL, Inc., **Research Triangle Park, NC**

Justification: To include the city and state.

Item 14

Section 10.0 References , page 21 which reads:

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20**, 482-491.

EDSTAC (1998). Endocrine Disruptor Screening and Testing Advisory Committee, Final Report, Volume I.

Huber, P.J. (1967). The behavior of maximum likelihood estimates under nonstandard conditions. In: *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability* **1**, 221-233.

Levene, H. (1960). Robust tests for the equality of variance. In: *Contributions to Probability and Statistics* (I. Olkin, S.G. Ghurye, W. Hoeffding, W.G. Madow, and H.B. Mann, Eds.), Palo Alto, CA, Stanford University Press, pp. 278-292.

NRC (1996). *Guide for the Care and Use of Laboratory Animals*. Institute of Laboratory

Animal Resources, Commission on Life Sciences, National Research Council. Revised 1996.

O'Connor, J.C., J.C. Cook, S.C. Craven, C.S. Van Pelt, and J.P. Obourn (1996). An *in vivo* battery for identifying endocrine modulators that are estrogenic or dopamine regulators. *Fundam. Appl. Toxicol.* **33,** 182-195.

O'Connor, J.C., S.R. Frame, L.G. Davis, and J.C. Cook (1999). Detection of thyroid toxicants in a tier I screening battery and alterations in thyroid endpoints over 28 days of exposure. *Toxicol. Sci.* **51(1),** 54-70.

O'Connor, J.C., S.R. Frame, and G.S. Ladics (2002). Evaluation of a 15-day screening assay using intact male rats for identifying steroid biosynthesis inhibitors and thyroid modulators. *Toxicol. Sci.* **69**, 79-91.

O'Connor, J.C., S.R. Frame, and G.S. Ladics (2002). Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicol. Sci.* **69**, 92-108.

Royall, R.M. (1986). Model robust confidence intervals using maximum likelihood estimators. *International Statistical Review* **54**, 221-226.

RTI (2001). *SUDAAN User's Manual, Release 8.0.* Research Triangle Park, NC: Research Triangle Institute.

SAS Institute Inc. (1999a). *SAS® Language Reference: Concepts*, Version 8, Cary, NC: SAS Institute Inc. 554 pp.

SAS Institute Inc. (1999b). *SAS/STAT® Users' Guide*, Version 8, Cary, NC: SAS Institute Inc. 3884 pp.

SAS Institute Inc. (1999c). *SAS® Language Reference: Dictionary*, Version 8, Cary, NC: SAS Institute Inc. 1244 pp.

SAS Institute Inc. (1999d). *SAS® Procedures Guide*, Version 8, Cary, NC: SAS Institute Inc. 1643 pp.

SAS Institute Inc. (1999e). *SAS® Companion for the Microsoft Windows Environment*, Version 8, Cary, NC: SAS Institute Inc. 562 pp.

SAS Institute Inc. (2000). *SAS/STAT® Software: Changes and Enhancements, Release 8.1*, Cary,

NC: SAS Institute Inc. 554 pp.

Zeger, S. and K. Liang (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* **42**, 121-130.

Is hereby amended to read:

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20**, 482-491.

EDSTAC (1998). Endocrine Disruptor Screening and Testing Advisory Committee, Final Report, Volume I.

Federal Register 163 (Dec. 28, 1998), Notice 71542-71568.

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Huber, P.J. (1967). The behavior of maximum likelihood estimates under nonstandard conditions. In: *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability* **1**, 221-233.

Levene, H. (1960). Robust tests for the equality of variance. In: *Contributions to Probability and Statistics* (I. Olkin, S.G. Ghurye, W. Hoeffding, W.G. Madow, and H.B. Mann, Eds.), Palo Alto, CA, Stanford University Press, pp. 278-292.

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O'Connor, J.C., J.C. Cook, S.C. Craven, C.S. Van Pelt, and J.P. Obourn (1996). An *in vivo* battery for identifying endocrine modulators that are estrogenic or dopamine regulators. *Fundam. Appl. Toxicol.* **33,** 182-195.

O'Connor, J.C., S.R. Frame, L.G. Davis, and J.C. Cook (1999). Detection of thyroid toxicants in a tier I screening battery and alterations in thyroid endpoints over 28 days of exposure. *Toxicol. Sci.* **51(1),** 54-70.

O'Connor, J.C., S.R. Frame, and G.S. Ladics (2002). Evaluation of a 15-day screening assay using intact male rats for identifying steroid biosynthesis inhibitors and thyroid modulators. *Toxicol. Sci.* **69**, 79-91.

O'Connor, J.C., S.R. Frame, and G.S. Ladics (2002). Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicol. Sci.* **69**, 92-108.

Royall, R.M. (1986). Model robust confidence intervals using maximum likelihood estimators. *International Statistical Review* **54**, 221-226.

RTI (2001). *SUDAAN User's Manual, Release 8.0.* Research Triangle Park, NC: Research Triangle Institute.

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SAS Institute Inc. (1999c). *SAS® Language Reference: Dictionary*, Version 8, Cary, NC: SAS Institute Inc. 1244 pp.

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SAS Institute Inc. (1999e). *SAS® Companion for the Microsoft Windows Environment*, Version 8, Cary, NC: SAS Institute Inc. 562 pp.

SAS Institute Inc. (2000). *SAS/STAT® Software: Changes and Enhancements, Release 8.1*, Cary, NC: SAS Institute Inc. 554 pp.

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Zeger, S. and K. Liang (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* **42**, 121-130.